(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2003 (30.01.2003)

PCT

(10) International Publication Number
WO 03/008618 A2

(51) International Patent Classification[7]: C12P 19/18, 19/08, C12N 15/52, 9/10, 1/20, 1/21, C08B 37/02, A23L 1/054 // (C12P 19/18, C12R 1:225)

(21) International Application Number: PCT/NL02/00495

(22) International Filing Date: 22 July 2002 (22.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

01202752.0	20 July 2001 (20.07.2001)	EP
01202841.1	25 July 2001 (25.07.2001)	EP

(71) Applicant *(for all designated States except US)*: **NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUUR-WETENSCHAPPELIJK ONDERZOEK TNO** [NL/NL]; Schoemakerstraat 97, NL-2628 VK Delft (NL).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **VAN GEEL-SCHUTTEN, Gerritdina, Hendrika** [NL/NL]; Bosstraat 78, NL-3971 XG Driebergen (NL).

(74) Agent: **JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/008618 A2

(54) Title: NOVEL GLUCANS AND NOVEL GLUCANSUCRASES DERIVED FROM LACTIC ACID BACTERIA

(57) Abstract: The invention pertains to novel glucans capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, the glucan having an average molecular weight between 10 kDa and 1 GDa, consisting essentially of α(1,3)- and α(1,6)-linked anhydroglucose units (AGU) and to glucansucrases capable of producing these glucans from sucrose. The glucans have thickening and anti-corrosive properties. The glucans can be chemically modified.

Novel glucans and novel glucansucrases derived from lactic acid bacteria

[0001] The present invention is in the field of enzymatic production of biomolecules. The invention is particularly concerned with novel glucans derived from lactic acid bacteria, with novel glucosyl-transferases derived from such bacteria and with a process for production of new and useful glucans from sucrose.

Background of the invention

[0002] Several bacteria are known to produce exopolysaccharides, i.e. polysaccharides secreted into the culture medium. Well-known examples of bacterial exopolysaccharides include xanthan from *Xanthomonas campestris*, gellan from *Sphingomonas paucimobilis* and pullulan from *Aureobasidium pullulans*. Lactic acid bacteria known to produce exo-polysaccharides include *Leuconostoc mesenteroides* strains producing dextrans, α(1→ 6)-linked poly-anhydroglucose, and alternans i.e. poly-anhydroglucoses having alternating α(1→ 6) and α(1→ 3)-linkages, oral *Streptococcus* strains producing glucans responsible for dental plaque formation, and a particular *Lactobacillus reuteri* strain producing α(1,6)- and α(1,4)-linked anhydroglucose (Van Geel-Schutten, *et al.*, *Appl. Environ. Microbiol.* (1999) 65, 3008-3014). The properties of exopolysaccharides depend on the type of monosaccharide units, the type of linkages, the degree and type of branching, the length of the polysaccharide chain, the molecular weight and the conformation of the polymers.

[0003] Argüello-Morales *et al.* (*FEMS Microbiol. Lett.* **182** (2000) 81-85) describe an alternansucrase from *Leuconostoc mesenteroides* NRRL B-1355. Monchois *et al.* (*Gene* **182** (1996) 23-32; *FEMS Microbiol. Lett.* **159** (1998) 307-315) for instance describe two different dextransucrases from *Lc. mesenteroides* NRRL B-1299. A method for selecting *Leuconostoc mesenteroides* strains that produce a high proportion of alternan to dextran is described in US 5,789,209. The prior art does not disclose or suggest other lactic acid bacteria than *Leuconostoc* or *Streptococcus* that are capable of producing glucans having both α(1→ 6) and α(1→ 3)-linkages.

Summary of the invention

[0004] Several lactic acid bacteria strains were found, according to the invention, to be capable of producing a particular class of glucans. These glucans have in common that their anhydroglucose units (AGU) are linked α(1,3)- and/or α(1,6)-glucosidic bonds, i.e. they are α-glucans largely or completely devoid of α(1,4)-bonds. These glucans may be of

the alternan (alternating α(1,3) and α(1,6) linkages), mutan (mixed α(1,3) and α(1,6) linkages, usually α(1,3) predominant) or dextran (mainly α(1,6) linkages, some α(1,3)) type, or other type. The glucans can be produced from sucrose, using sucrase enzymes which are active in the lactic acid bacteria. They can be produced on a large scale and isolated in a commercially feasible way, as the glucans are produced outside the bacterial cell, or even in the absence of the bacteria, using isolated sucrase enzymes. The glucans are produced by food-grade strains and have interesting properties, such as prebiotic utility or thickening of water-based compositions.

[0005] The invention is concerned with these novel glucans, with the lactic acid bacterial, especially *Lactobacillus* strains and their enzymic proteins that produce these glucans from sucrose, as well as with methods for producing the glucans using the strains and/or their enzymes, with nucleotide sequences encoding these enzymic proteins which convert sucrose, with the use of the glucans as thickeners, prebiotics, anticorrosives, etc., and as starting materials for modified glucans.

Description of the invention

[0006] The invention pertains to *Lactobacillus* strains containing a glucosyltransferase (glucansucrase) capable of producing a glucan having at least 10 anhydroglucose units (AGU) having a backbone consisting essentially of α(1,3)- and/or α(1,6)-linked AGU, in the presence of sucrose. Such strains can be found among current sources of *Lactobacilli*, such as food sources, silage, mammalian samples etc. These strains containing the glucosyltransferases and producing the glucans can be identified by isolating *Lactobacillus* strains from these sources, growing them on sucrose and analysing the polysaccharide product using suitable analytical methods such as chromatography. The genes encoding these glucosyltransferases can be identified by amplifying nucleotide sequence fragments of the strain using primers based on known glucosyltransferase genes and retaining the positive strains (see examples). Several glucan-producing strains were isolated and identified from different sources and different *Lactobacillus* species, such as *Lb. reuteri, Lb. fermentum, Lb. sake* and *Lb. parabuechneri* or related species. The glucosyltransferases from these glucan-producing strains were also identified and, completely or partly, sequenced (see Examples).

[0007] The novel glucans of the invention are capable of being produced by glucosyl-transferase (glucansucrase) activity of a lactic acid bacterium on a sucrose donor substrate. The glucans have an average molecular weight between 10 kDa and 1 GDa, and

consist essentially of α(1,3)- and/or α(1,6)-linked anhydroglucose units (AGU), to which side-chains also consisting of α(1,3)- and/or α(1,6)-linked AGU may be attached.

[0008] In particular, the glucans according to the invention either comprise 15-80% of α(1,3)-linked AGU, 2-80%, especially 4-80% and more especially 15-80% of α(1,6)-linked and 2-25% of α-(1,3,6)-linked (branching) AGU, or 80-99% of α(1,6)-linked AGU and 1-20% of α(1,3)-linked or α-(1,3,6)-linked (branching) AGU, in particular 1-15% of α(1,3)-linked AGU and 5-15% of α(1,3)- and α(1,3,6)-linked units taken together. Thus, the invention covers a glucan having an average molecular weight of 50 kDa to 1 MDa and comprising 25-50%, especially 29-39% of α(1,3)-linked AGU, 20-45%, especially 30-40% of α(1,6)-linked AGU, 5-25%, especially 3-13% of α(1,3,6)-linked AGU and 6-30% of terminal AGU. Furthermore, the invention pertains to a glucan having an average molecular weight of 10-50 MDa and comprising 15-26% α(1,3)-linked AGU, 30-50% of α(1,6)-linked AGU, 5-20% of α(1,3,6)-linked AGU and 5-35% of terminal AGU. Also, in another embodiment the invention covers a glucan having an average molecular weight of 1-50 MDa and comprising 40-60% of α(1,3)-linked AGU, 2-20%, especially 2-12% of α(1,6)-linked AGU, 10-25% of α(1,3,6)-linked AGU and 10-30% of terminal AGU. In yet another embodiment, the invention comprises a glucan having an average molecular weight of 10-50 MDa and comprising 80-99%, especially 88-99% and more especially 90-99% of α(1,6)-linked AGU, or 80-90% of α(1,6)- and 1-10% of α(1,3)-linked AGU, the remainder being 1,3,6 linked and terminal AGU.

[0009] The invention also concerns the enzymes originating from lactic acid bacteria, or from recombinant sources, capable of producing the glucans described above starting from sucrose. The enzymes are new and they can be classified as glucansucrases or glucosyltransferases. Their partial sequence information is given below in SEQ ID No's 1-10. More complete sequence information is given in SEQ ID No's 11-22. Proteins according to the invention comprise an amino acid sequence exhibiting at least 70%, preferably at least 80%, most preferably at least 90%, amino acid identity with any one of the amino acid sequences of SEQ ID No. 2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 or of stretches of at least 221-224 amino acids thereof, or at least 100 contiguous amino acids exhibiting at least 80%, preferably at least 90%, amino acid identity with these sequences. Further preferred sequences are indicated in the description of the alignment figure given below.

[0010] The enzymes can be used as such for producing the glucans described above, or for producing oligosaccharides and polysaccharides having a similar α(1,3) and/or α(1,6) linked glucan structure. Their genes can also be incorporated in suitable host organisms, to produce alternative glucan-production systems. The invention also pertains to such recombinant, preferably food-grade microorganisms, e.g. bacteria, especially lactic acid bacteria, yeasts, fungi etc., containing the genes of the glucansucrases described above and being capable of expressing the glucansucrases.

[0011] The invention also pertains to a process of producing a glucan as described above. This glucan can be produced by a *Lactobacillus* strain as described above, or by a recombinant micro-organism expressing the glucosyltransferase according to the invention or by an isolated glucosyltransferase according to the invention and a suitable glucose source such as for instance sucrose. The glucosyltransferase may be isolated by conventional means from the culture of a glucosyltransferase-positive lactic acid bacterium, especially a *Lactobacillus* species, or from a recombinant organism expressing the glucosyltransferase gene.

[0012] The glucan and the gluco-oligosaccharides produced by the *Lactobacillus* strains can be recovered from the culture supernatant of *Lactobacillus* strains described above, containing the glucosyltransferase according to the invention. The glucan can comprise at least 20, up to about 100,000 α-anhydroglucose units with the unique structure described above.

[0013] The glucan-producing enzymes according to invention, or at least the most preferred ones, are constitutive in the Lactobacillus strains, in that they are always present. This is contrast to most glucan (dextran-) producing Leuconostoc strains of the prior art, wherein the enzymes are only expressed upon growth in the presence of sucrose. This allows a more efficient production of glucans by the microorganisms of the invention.

[0014] The glucans according to invention have a variety of useful properties. They are suitable as prebiotics, and thus they can be incorporated in nutritional or pharmaceutical compositions intended for improving the condition of the gastrointestinal tract. For this purpose, they can be used as such or in the form of their oligosaccharides. They can also be combined with other poly- or oligosaccharides, such as fructans, galactans, xylans, arabinans, mannans, indigestible glucans and hetero-oligosaccharides, or with probiotic micro-organisms, including the lactic acid bacteria from which the glucans originate, resulting in synbiotic compositions. The glucans and their shortened homologues are also

useful as bioactive agents, e.g. as immunomodulators, anti-ulcer agents and cholesterol-lowering agents.

[0015] The glucans are also useful as thickening agents. As such they can be incorporated in foodstuffs such as beverages, sauces, dressings, dairy products, in amounts of from 1 g/l to about 100 g/l, especially about 10 to 50 g/l.

[0016] The glucans of the invention are furthermore useful as anticorrosion agents, for example for the protection of ship hulls. For that purpose, they may be applied in the form of solutions or suspensions, by spraying, coating, dipping and other techniques known in the art of corrosion control.

[0017] The glucans can be used as such. They can also be modified by physical or chemical means. Suitable examples of chemical modification include oxidation, especially 2,3- or 3,4-oxidation using periodate or hypohalite, in glucans having α-1,6 linkages, or 6-oxidation using nitroxyls with peracid or hypohalite in glucans having α-1,3 linkages. Hypohalite oxidation resulting in ring-opened 2,3- or 3,4-dicarboxy-anhydroglucose units (see e.g. EP-A-427349), while periodate oxidation results in ring-opened 2,3- or 3,4-dialdehyde-anhydroglucose units (see e.g. WO 95/12619), which can be further oxidised to (partially) carboxylated units (see e.g. WO 00/26257). Nitroxyl-mediated oxidation using hypochlorite or a peracid results in 6-aldehyde- and 6-carboxy-anhydroglucose units (see e.g. WO 95/07303).

[0018] The oxidised glucans have improved water-solubility, altered viscosity and a retarded fermentability and can be used as metal-complexing agents, detergent additives, strengthening additives, bioactive carbohydrates, emulsifiers and water binding agents. They can also be used as starting materials for further derivatisation such as cross-linking and the introduction of hydrophobes. Oxidised glucans coupled to proteins can be used as emulsifiers and stabilisers. The oxidised glucans of the invention preferably contain 0.05-1.0 carboxyl groups, more preferably 0.2-0.8 carboxyl groups per anhydroglucose unit, *e.g.* as 6-carboxyl groups on 1,3-linked units.

[0019] When modified glucans with high proportion of carboxyl groups are desired, two oxidation processes can be combined or an oxidation can be combined with e.g. carboxymethylation (see below). Thus, an α-(1,3/1,6)-glucan having a degree of substitution (DS) for carboxyl groups between 0,3 and 1,0 can be conveniently prepared by first nitroxyl-mediated oxidation, resulting in 1,3-substituted units being oxidation to glucuronic acid units, followed by e.g. periodate and chlorite oxidation, resulting in 1,6-substituted units* being converted to ring-opened dicarboxy-substituted units. The order

of processes can also be inverted, or one oxidation process, such as nitroxyl-mediated 6-oxidation can be combined with carboxymethylation. Also, by appropriate adaptation of the oxidation processes mixed aldehyde-containing and carboxyl-containing polymers can be obtained.

[0020] Other useful modifications are alkylation, acylation, hydroxyalkylation, amino-alkylation, carboxyalkylation, phosphorylation, sulphatation, as well as physical and chemical crosslinking. Phosphorylation (see: O.B. Wurzburg (1986), Modified Starches: properties and uses. CRC Press Inc., Boca Raton, 97-112) can be achieved by dry heating glucans with a mixture of monosodium and disodium hydrogen phosphate or with tripoly-phosphate. The phosphorylated glucans are suitable as wet-end additives in papermaking, as binders in paper coating compositions, as warp sizing-agents, and as core binders for sand molds for metal casting. Acylation, especially acetylation or propionylation using acetic or propionic anhydride respectively, results in products suitable as bleaching assistants and for the use in foils. Acylation with e.g. alkenyl succinic anhydrides or (activated) fatty acids results in surface-active products suitable as e.g. surfactants, emulsifiers, and stabilisers. Crosslinking, e.g. by coupling oxidised derivatives, or by reaction with a crosslinking agent such as triphosphoric acid, epichlorohydrine or a dialdehyde, can be used to adjust the physical properties of the glucans, e.g. to enhance their water-binding or thickening capacities.

[0021] Hydroxyalkylation is commonly performed by base-catalysed reaction with alkylene oxides, such as ethylene oxide, propylene oxide or epichlorohydrin; the hydroxy-alkylated products have improved solubility and viscosity characteristics. Carboxy-methylation is achieved by reaction of the glucans with monochloroacetic acid or its alkali metal salts and results in anionic polymers suitable for various purposes including crystallisation inhibitors, and metal complexants. Amino-alkylation can be achieved by reaction of the glucans with alkylene-imines, halo-alkyl amines or amino-alkylene oxides, or by reaction of epichlorohydrine adducts of the glucans with suitable amines. These products can be used as cationic polymers in a variety of applications, especially as a wet-end additive in paper making to increase strength, for filler and fines retention, and to improve the drainage rate of paper pulp. Other potential applications include textile sizing and wastewater purification. The above mentioned modifications can be used either separately or in combination depending on the desired product. Furthermore, the degree of chemical modification is variable and depends on the intended use. If necessary 100% modification, i.e. modification of all anhydroglucose units can be performed. However,

partial modification, e.g. from less than 1 (e.g. 0.2) modified anhydroglucose unit per 100 units up to higher levels, will often be sufficient in order to obtain the desired effect.

[0022] Another suitable type of derivatives is formed by hydrolysates of the present glucans. Hydrolysis can be performed in a controlled manner in a way known per se, using e.g. dilute acid or glucanolytic enzymes, especially α-1,3-glucanases or α-1,6 glucanases. Hydrolysis results in polysaccharides of reduced chain length (degree of polymerisation, DP, of more than 20) or oligosaccharides (DP of less than 20).

[0023] The invention also relates to gluco-oligosaccharides containing the characteristic structure of the glucan described above. These can be produced using an isolated glucansucrase according to the invention or a *Lactobacillus* strain, or a recombinant micro-organism containing (a part of) a glucosyltransferase according to the invention. Gluco-oligosaccharides thus produced can be used as prebiotics and probiotics. The production of the gluco-oligosaccharides is different from the glucan synthesis reaction. In addition to sucrose, the substrate of the glucansucrase, an acceptor molecule such as maltose or lactose can be used as an acceptor, to synthesise oligosaccharides. Consecutive attachment of glucose units in a manner determined by the particular glucansucrase results in α(1,3)- and/or α(1,6)-linked gluco-oligosaccharides, the chain length of which can be determined by selecting the appropriate reaction conditions. Longer reaction times, higher sucrose levels and lower acceptor levels will usually result in relatively long chains, e.g. having a degree of polymerisation (DP) of more than 10, up to several hundreds if desired, while shorter reaction times, lower sucrose levels and higher acceptor levels will result in relatively short chains, e.g. with a DP from about 3 up to 10 or higher. Another way of producing gluco-oligosaccharides is by hydrolysis of the glucan described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as amylase, dextranase or pullulanase or by acid hydrolysis. The produced gluco-oligosaccharides contain at least one 1,6- or one 1,3-glucosidic link to be used as prebiotics.

[0024] The invention also relates to a probiotic or synbiotic composition containing a *Lactobacillus* strain capable of producing a glucan and/or gluco-oligosaccharide according to the invention. The strain may also produce another poorly digestible poly- or oligosaccharide, such as a fructan. The probiotic or synbiotic compositions of the invention may be directly ingested with or without a suitable vehicle or used as an additive in conjunction with foods. They can be incorporated into a variety of foods and beverages including, but not limited to, yoghurts, ice creams, cheeses, baked products

such as bread, biscuits and cakes, dairy and dairy substitute foods, confectionery products, edible oil compositions, spreads, breakfast cereals, juices and the like.

[0025] Furthermore, the invention pertains to a process of improving the microbial status in the mammalian colon comprising administering an effective amount of a *Lactobacillus* strain capable of producing a glucan and/or gluco-oligosaccharide according to the invention. Furthermore, a process of improving the microbial status of the mammalian colon comprising administering an effective amount of a glucan or gluco-oligosaccharide according to the invention is also a part of the present invention.

Examples

General

The various lactic acid bacterial strains were isolated form a variety of sources, including fermented foods, the gastrointestinal tract of various human or animal species, and silage.

Example 1: *Identification and nucleotide sequence of glucansucrase/glucosyltransferase genes from lactobacilli*

The glucansucrase genes were identified by amplification with PCR using degenerated primers (GTFrev, 5' ADRTC NCCRT ARTAN AVNYK NG 3' and GTFforw, 5'-GAYAAYWSNA AYCCNRYNGT NC-3'; N = A, C, G or T, Y = T or C, K = G or T, W = A or T, S = C or G, R = A or G), based on conserved amino acid sequences of different published glucansucrase genes. An amplification product with the predicted size of about 660 bp was obtained and cloned in *Escherichia coli* Top 10 using pCR-XL-TOPO (Invitrogen). Sequence analysis confirmed that part of a *gtf* gene had been isolated. The 660 bp amplified was used to design primers for inversed PCR. For inverse PCR chromosomal DNA was digested with 10 different enzymes ligated, yielding circular DNA molecules. PCR with the diverging primers with the circular ligation products as template yielded amplicons of various sizes, those products were cloned into pCR-XL-TOPO (Invitrogen) and sequenced (GATC, Konstanz, Germany). If necessary additional inverse PCR reactions were carried out to obtain the complete gene(s). Both strands of the entire glucansucrase genes were sequenced twice.

Example 2: *Isolation and identification of α-(1,6) glucan and a glucansucrase from Lactobacillus reuteri strain 180*

L. reuteri strain 180 was deposited as LMG P-18389 at the BCCM/LMG Culture Collection at Gent, Belgium. The strain was grown in 18 litres of MRS-s medium (in g per kg): yeast extract (22), sodium acetate trihydrate (5), sodium citrate dihydrate (2.42), ammonum chloride (1.32), dipotassium hydrogen phosphate (2), magnesium sulphate heptahydrate (0.2), manganese sulphate heptahydrate (0.05), sorbitan mono-oleate (1), vitamins (in mg per kg: B1: 14.4, B2: 3.6, B3: 72, H 0.216), sucrose (100), tap water

(remainder), for 21 h at 37°C under anaerobic conditions (pH 5.5). See also: Van Geel-Schutten et al., Appl. Microbiol. Biotechnol. (1998) 50, 697-703. During growth, 13 g/l polysaccharide was produced. This polysaccharide was isolated as described in the reference cited above. The monosaccharide composition of the polysaccharide was determined by hydrolysis of the soluble part of the polysaccharide and high-performance anion-exchange chromatography. It was characterised as a glucan. This glucan was not formed when the strain was grown on glucose instead of sucrose. Methylation analysis (Van Geel-Schutten et al. 1999) revealed the presence of 17-24% α(1,3)-linked glucosyl units, 34-44% of α(1,6)-linked glucosyl units, 7-15% of α(1,3,6)-linked glucosyl units and 7-35% of terminal glucosyl units. The average molecular weight of the glucan was determined to be 3.6×10^7 Da and the Rg was 45 nm.

The average molecular weight of the polysaccharide was established using the SEC-MALLS system: 0.0522 g of the glucan was dissolved in 10 ml DMSO/water (90/10) and heated for 1 hour at 80°C, filtered through a 0.45µm filter and injected on the SEC-MALLS system and analysed using the following conditions:

Eluent:	DMSO/water (90/10) with 0.1 M $NaNO_3$
Flow rate:	0.5 ml/min
Injection volume:	0.247 ml
Column:	PLgel Guard, mixed-A and mixed-D
Temperature:	90°C

Detection: MALLS (DAWN-DSP), 50°C, A_2=0, dn/dc=0.074, F2 cell, RI; SDS PAGE followed by PAS-staining (Van Geel-Schutten et al. 1999) revealed the presence of an extracellular sucrase with a molecular weight of about 190 kDa. Part of the gene encoding the sucrase enzyme was isolated using PCR techniques and sequenced. On the deduced amino acid sequence of the fragment, high homologies were found with other glucan-sucrases. This partial sequence information is given in SEQ ID No. 1 (DNA) and 2 (protein). Full sequence information is given in SEQ ID No's. 11 and 12.

The glucan produced by *L. reuteri* strain 180 has been tested for application on ship hulls for the prevention of corrosion (see Example 8).

Example 3: Isolation and identification of α-(1,6/1,3) glucan and a glucansucrase from Lactobacillus reuteri strain ML1

L. reuteri strain ML1, deposited as LMG P-20347 at the BCCM/LMG Culture Collection at Gent, Belgium, was grown overnight under anaerobic conditions at 37°C on MRS supplemented with sucrose (see Example 2). The cells were removed by centrifugation and two volumes of ethanol were added to the supernatant. The precipitated poly-saccharides were harvested by centrifugation and resuspended in 2-3 liters of demi water and precipitated again with two volumes of ethanol. The glucan produced by this strain (7 g) was characterised by methylation analysis and monosaccharide composition analysis as

described in Example 2. The polymer was found to consist of 48-53% of α(1-3) linked glucosyl units, 3-8% of α(1-6) linked glucosyl units, 12-20% of α(1-3-6) linked glucosyl units (branching units) and 20-30% of 1-linked (terminal) glucose units. The glucans were not produced during growth on glucose. The average molecular weight of the polysaccharide was established to be $7.6x10^6$ Da using the SEC-MALLS system as described in example 2. These were the first examples of the production of mutan-like polymers by lactobacilli. The glucan produced by *L. reuteri* strain ML1 has been tested for application as anticorrosive agent and showed excellent utility for the prevention of corrosion e.g. on ship hulls.

SDS PAGE followed by PAS-staining (Van Geel-Schutten et al. 1999) revealed the presence of an extracellular sucrase with a molecular weight of about 190 kDa. It was found that this strain produces two glucansucrases. Sequence information for these sucrase is given in SEQ ID No's 13 and 14 (ML1) and 15 and 16 (ML4).

Example 4: Isolation and identification of α-(1,6/1,3) glucan and a glucansucrase from Lactobacillus strain LB 33.

A new *Lactobacillus* strain was obtained and was deposited as LMG P-20349. The strain was identified by 16S rRNA to be most closely related to *Lactobacillus parabuchneri*. The strain grown overnight on MRS supplemented with sucrose under anaerobic conditions at 37°C (see Example 2). 420 gram of glucan was produced. The glucan produced by this strain is not produced during growth on glucose.

Methylation analysis (see Example 2) revealed that the polymer consists of equal amounts of 29-39% of α(1-3) linked glucosyl units, 30-40% of α(1-6) linked glucosyl units, 3-13% of α(1-3-6) linked glucosyl units (branching units) and 15-30% of 1-linked (terminal) glucose units.

The average molecular weight of the polysaccharide was established to be $2x10^5$ Da, using the SEC-MALLS system as described in Example 2.

By PCR with degenerated primers part of a sucrase type of glucosyl-transferase could be isolated indicating that the glucan is produced by a sucrase. This confirms the result that the glucan is produced during growth on sucrose and not on glucose. Part of the sucrase encoding gene was sequenced. On the deduced amino acid level high homologies were found with alternan sucrase from *Leuconostoc mesenteroides*. This indicates that the enzyme responsible for the glucan synthesis in *L. brevis* is the first alternan sucrase found in other bacteria than *Leuconostoc*. This partial sequence information is given in SEQ ID No. 3 (DNA) and 4 (protein). Full sequence information is given in SEQ ID No's. 17 and 18, respectively.

The glucan produced by this strain has thickening properties.

Example 5: *Isolation and identification of α-(1,6) glucan and a glucansucrase from Leuconostoc strain 86*

A new strain was obtained from silage and was deposited as LMG P-20350. The strain was identified by 16S rRNA to be a new *Leuconostoc* strain, most closely related to *Leuconostoc citreum*. The strain grown overnight on MRS supplemented with sucrose under anaerobic conditions at 37°C (see Example 2). 416 gram of glucan was produced. Methylation analysis of the glucan obtained revealed that more than 90 % of the glucose units was linked through an α (1,6) bond, identifying the polysaccharide as a dextran. The molecular weight of the glucan (determined as described in Example 2) was 3-4 x 10^7 Da and the Rg was 40 nm. The glucan is not produced during growth on glucose.

By PCR with degenerated primers 3 different fragments with part of a sucrase type of glucosyl-transferase could be isolated indicating that the glucan is produced by a sucrase and that possibly 3 sucrases are present in this strain. This confirms the result that the glucan is produced during growth on sucrose and not on glucose. Part of the sucrase encoding gene was sequenced. On the deduced amino acid level high homologies were found with DSRC and DSRB (fragment 1), alternan sucrase (fragment 2) and DSRA (fragment 3) from *Leuconostoc mesenteroides*. The sequence information is given in SEQ ID No's 5-10. *Leuconostoc citreum*, to which this new strain is most closely related, is not reported to produce dextran. The glucan produced by strain 86 has thickening properties.

Example 6: *Identification of α-(1,6/1,3) glucan and a glucansucrase from Lactobacillus sake KG 15*

Strain KG 15 was obtained from silage and was deposited as LMG P-21583. It was identified by 16S rRNA as *L. sake*. The strain was grown and the polysaccharide was recovered as described in example 2. The molecular weight of the polysaccharide was determined to be 4,7 10^7 Da (SEC MALLS) and the Rg was 92 nm. Methylation analysis (GC) revealed that the glucan produced by this strain is a largely linear dextran containing 4 % terminal glucose units, 86% of α(1,6) linked glucosyl units, 2% of α (1,3) linked glucosyl units and 8% α (1,3,6) disubstituted glucose units (branching points). The glucansucrase of this strain was sequenced (see SEQ ID No. 19 and 20).

Example 7: *Identification of α-(1,6/1,3) glucan and a glucansucrase from Lactobacillus fermentum KG 3*

Strain KG 3 was obtained from silage and was deposited as LMG P-21584. It was identified by 16S rRNA as *L. fermentum*. The strain was grown and the polysaccharide was recovered as described in example 2. The molecular weight of the polysaccharide was determined to be 2,4 10^7 Da (SEC MALLS) and the Rg was 107-119 nm. Methylation analysis (GC) revealed that the glucan produced by this strain is a largely linear dextran containing 3% terminal glucose units, 84% of α(1,6) linked glucosyl units,

8% of α (1,3) linked glucosyl units and 5% α (1,3,6) disubstituted glucose units (branching points). The glucansucrase of this strain was sequenced (SEQ ID No's 21 and 22).

Example 8: Anticorrosion properties of glucans

Plain carbon steel sheets of 1 cm^2 embedded in an epoxy matrix were exposed to a slightly corrosive medium (150 ml of 0.1 M $LiClO_4$) with or without the addition of a bacterial polysaccharide (0.2 g) for several days. The sheets were then examined visually and electrochemically from time to time. The corrosion potential (E_{corr} in mV with reference to Ag/AgCl) and polarisation resistance (R_p in $k\Omega/cm^2$) are both a measure of the anti-corrosion effect. After an initial adaptation of 3-10 hours, these parameters attained a stable value. The experiments were carried with a heteropolysaccharide from *Lactobacillus sake*, and a homopolysaccharide of the invention (from LB 180 according to example 4), as well as without polysaccharide. The results are summarised in the table below. It follows that the anti-corrosion properties of the glucan of the invention are superior. It was found that the homopolysaccharide of ML 1 (example 3) has at least equal anticorrosion performance as the LB 180 polysaccharide.

Table: Corrosion experiments

organism	type of poly-saccharide	aspect of treated sheet	E_{corr} (mV vs. Ag/AgCl)	R_p ($k\Omega/cm^2$)
control	-	corrosion	-700	1.5
Lb. sake	hetero-polysaccharide	localised corrosion	-600	4.5
Lb. 180	α-glucan	thin black layer	-200	70

Example 9: Modification of α-1,3/1,6-glucan by oxidation

One gram (6.15 mmol of anhydroglucose units) of the α-1,3/1,6-glucan produced by strain LB 33 (example 4) is resuspended in 100 ml water. Next, 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO; 0.01 g, 0.065 mmol) and sodium bromide (100 mg, 1 mmol) are added and the suspension is cooled to 0°C. The reaction can also be performed without bromide. A solution of hypochlorite (3 ml, 15% solution, 6.3 mmol) of pH 10.0 (0°C) is added. The pH is kept constant by addition of 0.1M NaOH. After 1 hr, the solution is poured into 150 ml 96% ethanol, causing the product to precipitate. The white precipitate is centrifuged, resuspended in ethanol/water (70/30 v/v) and centrifuged again. Next, the precipitate is resuspended in 96% ethanol, centrifuged and dried. The uronic acid content is determined by means of the uronic acid assay according to Blumenkrantz and Abdoe-Hansen (*Anal. Biochem.* 54 (1973), 484). A calibration curve was generated using polygalacturonic acid (5, 10, 15 and 20 μg). With this calibration curve the uronic

acid content in a sample of 20 μg of the product is determined. The major part of 6-hydroxyl groups have been oxidised to carboxyl groups.

Example 10: *Construction of plasmids for expression of the glucansucrase genes in E. coli.*

Two primers were designed with appropriate restriction sites; the C-terminal primer contained in all cases a His-tag. The PCR products were first cloned in pCR-XL-TOPO. The PCR products were removed from pCR-XL-TOPO using the appropriate enzymes and ligated in the appropriate sites of an expression vector (e.g pET15b (Novagen)).

For the expression of part of the glucosyltransferase gene of LB 180 (for better expression, the N-terminal region encoding the N-terminal variable domain of the glucansucrase,was not cloned) in *E. coli*, a PCR reaction was performed using Forw180 (5'-GATGCAT**GAG CTC**CATGG CATTAACGGC CAACAATATT ATTATTGACC C-3') containing *Sac*I (bold) and *Nco*I (underlined) sites, and Rev180 (5'-ATATCGAT**GG GCCCC**GGATC CTATTAGT*GA TGGTGATGGT GATGTT*TTTG GCCGTTTAAA TCACCAGGTT TTAATGG-3'), containing *Apa*I (bold), *Bam*HI (underlined) and a 6x His-tag (italics) as primers. The PCR product was cloned in pCR-XL-TOPO. The PCR product was removed from pCR-XL-TOPO using *NcoI*/*Bam*HI and ligated in the coresponding sites of pET15b (Novagen). The resulting plasmid (pET15b180) containing part of the glucansucrase gene of 704 amino acids encoding a glucansucrase without the variable N-terminal domain was transformed to *E. coli* Bl21 DE3 star (Invitrogen).

Cells of *E. coli* harbouring the pET15b180 were harvested by centrifugation after 16 h of growth under aerobic conditions at 37 °C. The pellet was washed with 50 mM sodium acetate buffer pH 5.5 containing 1 mM $CaCl_2$ and 1% (v/v) Tween 80 and the suspension was centrifuged again. Pelleted cells were resuspended in with 50 mM sodium acetate buffer pH 5.5 containing 1 mM $CaCl_2$ and 1% (v/v) Tween 80, and 7.2 mM β-mercapto-ethanol. Cells were broken by sonication and cell debris and intact cells were removed by centrifugation for 15 minutes at 4 °C at 14,000 rpm (Eppendorf). The resulting cell free extract was used as enzyme source to produce high molecular weight glucans from sucrose in 50 mM sodium acetate buffer pH 5.5 containing 1 mM $CaCl_2$ and 1% (v/v) Tween 80 and 10 g/l sucrose. After 16 hours of incubation, the glucans were isolated using ethanol precipitation. When cell free extracts of *E. coli* Bl21 DE3 star (Invitrogen) harbouring the plasmid pET15b (without insert) were used as enzyme source, no glucans were produced from sucrose.

Sequence information

SEQ ID No's 1 and 2 give the nucleotide and amino acid sequence, respectively, of a part of the glucansucrase from strain Lb180 as originally determined (Example 2). The partial

sequence shows 53% (199/223) sequence identity and 68% similarity with dextransucrase DSRB742 of *Leuconostoc mesenteroides (Lc. mes.)*, with 2 gaps (between amino acids F172 and N173), and 52% identity with some other dextransucrases and alternansucrases of *Lc. mes.*

SEQ ID No's 3 and 4 give the nucleotide and amino acid sequence, respectively, of a part of the glucansucrase from strain Lb 33 as originally determined (Example 4). The partial sequence shows 63% (143/224) sequence identity and 75% similarity with dextransucrase DSRB742 of *Lc. mes.* with 1 gap.

SEQ ID No's 5 and 6 give the nucleotide and amino acid sequence, respectively, of a part of a glucansucrase (86-1) from strain Lc 86 (Example 5). The partial sequence shows 98% (219/223) sequence identity and 99% similarity with dextransucrase DSRB742 of *Lc. mes.*

SEQ ID No's 7 and 8 give the nucleotide and amino acid sequence, respectively, of a part of another glucansucrase (86-5) from strain Lc 86 (Example 5). The partial sequence shows 55% (123/223) sequence identity and 68% similarity with dextransucrase DSRB742 of *Lc. mes.*, with 2 gaps (between amino acids M128 and R129 and between D162 and H163), and 51-56% identity with some other dextransucrases and alternansucrases of *Lc. mes.*.

SEQ ID No's 9 and 10 give the nucleotide and amino acid sequence, respectively, of another glucansucrase (86-8) from strain Lc 86 (Example 5). The partial sequence shows 61-68% sequence identity and 74-78% similarity with dextransucrases and alternansucrases (including dextransucrase DSRB742)of *Lc. mes.*

SEQ ID No's 11 and 12 give the nucleotide and amino acid sequence, respectively, of the glucansucrase of strain Lb180 (Example 2). The sequence shows 1322/1768 (74%) sequence identity and 1476/1768 (82%) similarity with 15/1768 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372. The –35 and –10 sites TTGAAA and TATAA are located at nucleotide positions 561 and 599, respectively. The ribosome binding site (RBS) GAAGGAG is at 574 and the start codon ATG at 587. Inverted repeats AAGCAGCTC and GAGCTGCTT are at 6025 and 6051. Possible stop codons (TAA, TAG, TGA) are indicated with an * (5963).

SEQ ID No's 13 and 14 give the nucleotide and amino acid sequence, respectively, of the glucansucrase I from strain ML1 (Example 3). The sequence shows 1327/1775 (74%) sequence identity and 1465/1775 (81%) similarity with 17/1775 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 43-44% sequence identity and 57-58% similarity with dextransucrases of *Lc. mes.* and 47% sequence identity and 61% similarity with an alternansucrases of *Lc. mes.* The RBS AAGGAGA is at 31 and the start codon ATG is at 43. A stop codon TAG is at 5356.

SEQ ID No's 15 and 16 give the partial nucleotide and amino acid sequence, respectively, of a second glucansucrase from strain ML1 (ML4) (Example 3). The sequence shows

301/817 (36%) sequence identity and 427/817 (51%) similarity with 12/817 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 38% sequence identity and 53% similarity with glucosyltransferase of *Streptococcus mutans*.

SEQ ID No's 17 and 18 give the partial nucleotide and amino acid sequence, respectively, of the glucansucrase from strain LB 33 (Example 4). The sequence shows 59% sequence identity and 71% similarity with several known dextransucrases of *Lc. mes.* and 53% sequence identity and 67% similarity with other known dextransucrases (including dextransucrase DSRB742) of *Lc. mes.*

SEQ ID No's 19 and 20 give the nucleotide and amino acid sequence, respectively, of the glucansucrase from Lb. strain KG 15 (Example 6). The sequence shows 496/1111 (44%) sequence identity and 637/1111 (56%) similarity with 71/1111 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 57-59% sequence identity and 70% similarity with several dextransucrases (including dextransucrase DSRB742) of *Lc. mes.* The –35 and –10 sites *TTGGAC and TATTAT are* located at nucleotide positions 477 and 502, respectively. The RBS GAAAGGA is at 593 and the start codon ATG at 608. A stop codon TAG is 5393. Inverted repeats AAAACAACCCCC and GGGGTTGTTTTT are at 5497 and 55 31 (-10.7 kcal/mole).

SEQ ID No's 21 and 22 give the partial nucleotide and amino acid sequence, respectively, of the glucansucrase from Lb. strain KG 3 (Example 7). The sequence shows 58 sequence identity and 71% similarity with known dextransucrases (including dextransucrase DSRB742) of *Lc. mes.*.

Description of the figure

Figure 1 depicts an amino acid sequence alignment of glucosyltransferases (GTF) according to the invention. It shows the partial sequences of the GTF of Lb 180 (first line, starting with amino acid 216 of SEQ ID No. 12); GTF of ML1 (second line, starting with amino acid 15 of SEQ ID No. 14), GTF of Lb 33 (third line, starting with amino acid 222 or 243 of SEQ ID No. 18); GTF of KG15 (fourth line, starting with amino acid 567 of SEQ ID No. 20) and GTF of KG3 (fifth line, starting with amino acid 1 (LMAAF) of SEQ ID No. 22); and a GTF according to the invention of a *Lb. reuteri* strain "104" (sixth line, 1 (WPNTV) – 525). The alignment is not necessarily the best fit according to automated alignment programs, but is intended to define the enzymes of the invention.

The invention not only covers amino acid sequences shown in this figure, but also sequences wherein amino acids of a given sequence in the figure are exchanged with the corresponding amino acids (including gaps) of another sequence of the figure. This applies to stretches of at least 100 amino acids having at least 80%, preferably at least 90% identity with any of the sequences of the figure, or of the sequences listings given separately. It especially applies to the stretch of amino acids between the consensus peptides DNSN and YYGD (from 1202 to 1422 of SEQ ID No 12). Especially preferred

are sequences comprising the active core of the enzymes, which are present between the consensus peptides INGQ and VPDQ (from 957 to 1724 of SEQ ID No 12), with preferably at least 70% identity with any one of the core sequences given. A preferred non-identity with a given sequence is an exchange with the corresponding amino acids of another sequence. Especially preferred sequences are those where an amino acid at a given position is shared between at least 2, in particular at least 3, of the sequences of the figure. Most preferred are those sequences in which one of those consensus sequences is that of the GTF of Lb180, ML1 or Lb33 (first three lines). The N-terminal part upstream of the core (shown in the figure for GTF 180 and GTF ML1 only), or the C-terminal part downstream of the core (not shown in the figure) may be wholly or partly present or may be absent.

Claims

1. A process of producing a glucan having at least 10 anhydroglucose units, having a backbone consisting essentially of α(1,3)- and/or α(1,6)-linked anhydroglucose units (AGU), comprising subjecting sucrose to the activity of a glucosyltransferase produced by a *Lactobacillus* strain capable of producing α(1,3)- and/or α(1,6)-linked glucans, or to the *Lactobacillus* strain capable of expressing the glucsylfransferase.

2. A *Lactobacillus* strain capable of producing, in the presence of sucrose, a glucan having at least 10 anhydroglucose units (AGU) having a backbone consisting essentially of α(1,3)- and/or α(1,6)-linked AGU.

3. A glucan capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, the glucan having an average molecular weight between 10 kDa and 1 GDa, especially between 10kDa and 50 MDa, and having a backbone consisting essentially of α(1,3)- and α(1,6)-linked anhydroglucose units (AGU).

4. A glucan according to claim 3, which is capable of being produced by glucosyl-transferase activity of a *Lactobacillus* species.

5. A glucan according to claim 4, comprising 15-80% of α(1,3)-linked AGU, 2-80% of α(1,6)-linked AGU, and 2-25% of α-(1,3,6)-linked AGU.

6. A glucan according to claim 5, having an average molecular weight of 50 kDa - 1 MDa and comprising 30-45% of α(1,3)-linked AGU, 30-45% of α(1,6)-linked AGU, and 3-13% of α(1,3,6)-linked AGU.

7. A glucan according to claim 5, having an average molecular weight of 10-50 MDa and comprising 15-26% α(1,3)-linked AGU, 30-50% of α(1,6)-linked AGU, 5-20% of α(1,3,6)-linked AGU.

8. A glucan according to claim 5, having an average molecular weight of 1-50 MDa and comprising 45-60% of α(1,3)-linked AGU, 4-10% of α(1,6)-linked AGU, and 10-20% of α(1,3,6)-linked AGU.

9. A glucan capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, having an average molecular weight of 10-50 MDa and comprising 80-99% of α(1,6)-linked AGU and 0-15% of α(1,3)-linked AGU.

10. A protein having glucosyltransferase activity, capable of producing, in the presence of sucrose, a glucan according to any one of claims 3-9.

11. A protein according to claim 10, comprising an amino acid sequence of at least 100 amino acids exhibiting at least 70%, preferably at least 80%, amino acid identity with any one of the amino acid sequences of SEQ ID No. 2, 4, 8, 10, 12, 14, 16, 18, 20 and 22, and/or having a stretch of 100 amino acids having at least 80%, preferably at least 90%, amino acid identity with any one of the said amino acid sequences, or having at least 99% amino acid identity with the amino acid sequence of SEQ ID No. 6, and/or having a stretch of 100 amino acids having 100% amino acid identity with the amino acid sequence of SEQ ID No. 6.

12. A nucleic acid sequence encoding a protein according to claim 11.

13. A recombinant host cell containing one or more copies of a nucleic acid construct comprising a nucleic acid sequence according to claim 12 and capable of expressing a protein having glucosyl-transferase activity.

14. A *Lactobacillus* strain, capable of producing a glucan according to any one of claims 3-9, especially a *Lactobacillus* strain corresponding to strain 33, 180 or ML1 as described herein.

15. A *Leuconostoc* strain, capable of producing a glucan according to claim 9, especially a *Leuconostoc* strain corresponding to strain 86, deposited under accession number LMG P-20350.

16. A chemically modified glucan, which is obtained by 2,3-oxidation, 6-oxidation, phosphorylation, acylation, alkylation, hydroxyalkylation, carboxymethylation, amino-alkylation of one or more AGU of a glucan according to any one of claims 3-9.

17. Use of a glucan according to any one of claims 3-9, as a thickener.

18. Use of a glucan according to any one of claims 3-9, as a prebiotic and/or as a bioactive agent.

19. Use of a glucan according to any one of claims 3-9, as an anti-corrosion agent.

20. Use of a *Lactobacillus* bacterium capable of producing a glucan according to any one of claims 3-9, as a probiotic agent, or together with an indigestible glucan, as a synbiotic agent.

FIG. 1 SEQUENCE ALIGNMENT

```
216  MEIKKHFKLYKSGKQWVTAAVATVAVSTALLYGGVAHADQQVQSSTTQEQTSTVNADTTK
 15  MEIKKHFKLYKSGKQWVTAAVATVAVSTALLYGGVAHADQQVQSSTTQDQTSTVNTNTTK

276  TVNLDTNTDQPAQTTDKNQVANDTTTNQSKTDSTSTTVKNPTFIPVSTLSSSDNEKQSQN
 75  TIAADTNADQPAQTADKNQAASNDTTNQSKTDSTSTTVKNLTSTPVSTLPSTDNEKQNQN

336  YNKPDNGNYGNVDAAYFNNNQLHISGWHATNASQGTDSRQVIVRDITTKTELGRTNVTNN
135  YNKHDNGNYGNIDTAYFSNNQLHVSGWNATNASQGTNSRQIIVRDITTNNELGRTDVTNN

396  VLRPDVKNVHNVYNADNSGFDVNINIDFSKMKDYRDSIEIVSRYSGNGKSVDWWSQPITF
195  VARPDVKNVHNVYNADNSGFDINVNIEFSKMKDYRDSIEIVSRYSGNGKSIDWWSQPITF

456  DKNNYAYLDTFEVKNGELHATGWNATNKAINYNHHFVILFDRTNGKEVTRQEVRDGQSRP
255  DKNNYAYLDTFEVKNGELHATGWNATNSAINYNHHFVILFDQTNGKEVARQEVREGQSRP

516  DVAKVYPQVVGANNSGFDVTFNIGDLDYTHQYQILSRYSNADNGEGDYVTYWFAPQSIAP
315  DVAKVYPQVVGADNSGFDVTFNIGNLDYTHQYQVLSRYSNSDNGEGDNVTYWFNPQSIAP

576  ANQSNQGYLDSFDISKNGEVTVTGWNATDLSELQTNHYVILFDQTAGQQVASAKVDLISR
375  ANQSNQGYLDSFDISKNGEVTVTGWNATDLSELQNNHYVILFDQTAGKQVASAKADLISR

636  PDVAKAYPTVKTAETSGFKVTFKVSNLQPGHQYSVVSRFSADENGNGNDKRHTDYWYSPV
435  PDVAKAYPTVKTAANSGFKVTFKVNDLQPGHQYSVVSRFSADENGNGNDKRHTDYWFSPV

696  TLNQTASNIDTITMTSNGLHITGWMASDNSINEATPYAIILNNGREVTRQKLTLIARPDV
495  TLNQNASNIDTITMTSNGLHIGSWMASDNSINETTPYAIILNNGKEVTRQKMSLTARPDV

756  AAVYPSLYNSAVSGFDTTIKLTNAQYQALNGQLQVLLRFSKAVDGNPNGTNTVTDQFSKN
555  AAVYPSLYNSAVSGFDTTIKLTNDQYQALNGQLQVLLRFSKAADGNPSGDNTVTDQFSKN

816  YATTGGNFDYVKVNGNQIEFSGWHATNQSNDKNSQWIIVLVNGKEVKRQLVNDTKDGAAG
615  YATTGGNFDYVKVNGNQVEFSGWHATNQSNDKDSQWIIVLVNGKEVKRQLVNDTKEGAAG

876  FNRNDVYKVNPAIENSIMSGFQGIITLPVTVKDENVQLVHRFSNDAKTGEGNYVDFWSEV
675  FNRNDVYKVNPAIENSSMSGFQGIITLPVTVKNENVQIVHRFSNDAKTGEGSHVDFWSEV

936  MSVKDSFQKGNGPLNQFGLQTINGQQYYIDPTTGQPRKNFLLQNGNDWIYFDKDTGAGTN
735  MPVKDSFQKGNGPLKQFGLQTINGHQYYIDPMTGQPRKNFLLQNGNDWLYFDNETGEGTN
222  VNGKIYFVGDNGQVKKNFTAIINGQSLYFNKTTGELASNDVQYENGLVKINDV
567                       QTIAGKTYYFDKD GHLRKGYSTIIDNQLYYFDLKTGESVS

996  ALKLQFDKGTISADEQYRRGNEAYSYDDKSIENVNGYLTADTWYRPKQILKDGTTWTDSK
795  ALKRQFDGGTISADSQYRKGNEAYGYDNKSIENVDGFLTADTWYRPKQILKW TTWTDSK
275                       HNAAYSIDP?GFTNVNGFLTANSWYRPKYIYKDGQKWVEST
607  TTTSNFKSGLTSQTDDTTPHNSAVNMSKDSFTTVDGFLTAESWYVPKDIQTSATDWRAST

1056 ETDMRPILMVWWPNTVTQAYYLNYMKQYGNLLPASLPSFSTDADSAELNHYSELVQQNIE
 854 ETDMRPLLMVWWPNTVTQAYYLNYMKQHGNLLPANLPFFNSDADPLELNYYAEIVQQNIE
 316 SQDMRPLLMTWWPDKNTQVAYLQYM QKMGILPADVTISSQTNQSVLTKESF ITQAEIE
 666 PEDFRPIMMTWWPTKQIQAAYLNHMVSEG LLSSDKKFSATD DQTLLNQAAHAVQLQIE
(0)
   1              WPNTVTQAYYLNYMKQHGNLLPASLPFFNADADPAELNHYSEIVQQNIE
```

```
1116 KRISET GSTDWLRTLMHEFVTKNSMWNKDSENVDYGGLQLQGGFLKYVNSDLTKYANSDW
 914 KKISQT GNTDWLRTLMHEFVSNNTMWNKNSENEDFGGLQLQGGFLKYVNSDKTPNANSNW
 374 KQIGVTNGNTDWLKKDISDFVNSQPNWNIDSEAKGTDH  LQGGALLYVNNKLTPYANSDY
 725 LKIQQT KSVEWLRTTMHNFIKSQPGYNVTSETPSNDH  LQGGALSYINSVLTPDANSNF
   1                LMAAFVVTQPQWNKTSEDVNDDH  LQGGALTFENNGDT DANSDY
  50 KRISET GNTDWLRTLMHEFVTKNSMWNKDSENVDYGGLQLQGGFLKYVNSDLTKYANSDW

1176 RLMNRTATNIDGKNY        GGAEFLLANDIDNSNPVVQAEELNWLYYLMNFGTITGN
 974 RIMGRQPANIDGNGP        IGSEFLLANDVDNSNPVVQAEQLNWLHYLLNFGTITAN
 433 RLLNRTLTNQQGQVKDTS  KQGGYEMLLANDVDNSNPVVQAEQLNWLYYMMNIGSITAN
 783 RLMNRNPTQQDGTRHYNTDTSEGGYELLLANDVDNSNPVVQAEQLNWLYFLTHFGEIVKN
  44 RLMNRTPTNQTGERLYHIDDSLGGYELLLANDVDNSNPQVQAEQLNWLYYLMHFGDITAD
 110 RLMDRTATNIDGKNY        GGAEFLLANDIDNSNPVVQAEELNWLYYLMNFGTITGN

1229 NPEANFDGIRVDAVDNVDVDLLSIARDYFNAAYNMEQSDASANKHINILEDWGWDDPAYV
1027 DPDANFDSIRVDAVDNVDADLLDIAGDYFNAVYHSQSNDKIANAHINILEDWGGQDPYYT
 491 DPTANFDGYRVDAVDNVDADLLNIAADYAKAYKTN QSDANANKHLSILEDWDNNDPAYI
 843 DPSANFDSVRVDAVDNVDADLLNITAAYFRDVYGVDKNDLTANQHLSILEDWGHNDPLYV
 104 DPDANFDAIRIDAVDNVDADLLQLAAQYFRDAYGMATTDATSNKHLSILEDWSHNDPAYM
 163 NPEANFDGIRVDAVDNVDVDLLSIARDYFNAAYNMEQSDANANKHINILEDWGWDDPAYV

1289 NKIGNPQLTMDDRLRNAIMDTLSGAPDKNQALNKLITQSLVNRANDN TENAVIPSYNFV
1087 QSIGTPQLSMDYNFSTIRSVLASNTASMTD    IIKNSLVNRSLDN AENVSIPNYSFI
 551 KAHGNNQLTMDFPAHLAIKYSLNMPVSQRSGLEPELTTSLVNRTGDDSTENVAQPNYTFI
 903 KDHGSDQLTMDDYMHTQLIWSLTKNPDNRSAMRRFMEYYLVDRAKDN TSDPAIPNYSFV
 164 QAHGNDQLTMDDYMHTQLIWSLTKPEAQRGTMARFMDFYLTNRANDD TENTAQPSYSFV
 223 NKIGNPQLTMDDRLRNAIMDTLSGAPDKNQALNKLITQSLVNRANDN TENAVIPSYNFV

1348 RAHDSNAQDQIRQAIQAATGKPYGE  FNLDDEKKGMEAYINDQNSTNKKWNLYNMPSAY
1142 RAHDNGSQDDIKRAISDVNNLPYGSK FNFEQEQKGIEAYIADQSNVNKKWNNYNIPSSY
 611 RAHDSEVQTIIAQIIKDKINPNSDGLTVTPDEISQAFKIYNADELKTDKQYTFYNMPSAY
 962 RAHDSEVQTVIGDIVAKLYPDVKNSL PSMEQLAAAFKVYDADMNSVNKKYTQYNMPAAY
 223 RAHDSEVQTVIAEIVTKLHPEAGNGLMPTEEQMAEAFKIYNADQKKAVKTYTHYNMPSAY
 282 RAHDSNAQDQIRQAIQAATGKPYGE  FNLDDEKKGMEAYINDQNSTNKKWNLYNMPSAY

1406 TILLTNKDSVPRVYYGDLYQDGGQYMEHKTRYFDTITNLLKTRVKYVAGGQTMSVDKN
1201 AIMLTNKDTVPRVYYGDLFTDGGQYMAQTTRYYPALTSLLKARIKYVAGGQTMSVDKN
 671 TILLTNKDTVPRVYYGDLYSDNGNYMSAHSPYYDAITTLLKTRMKYVSGGQNMRMQYMQG
1021 AMLLTNKDTIPRVYYGDMYTDDGQYMATKSPYYDAISALLKARIKYVAGGQTMAVDKH
 283 AMLLTNKDVIPRIYYGDLYTDDGQFMATKSPYFDAISTMLQARTKYVAGGQTMAVDQH
 340 TILLTNKDSVPRVYYGDLYQDGGQYMEHKTRYFDTITNLLKTRVKYVAGGQTMSVDKN

1464          GILTNVRFGKGAMNATDTGTDETRTEGIGVVISNNTNLKLNDGESVVLHMG
1259          NILTSVRFGKGAMNPTDMGDSLTRTSGVGVVISNNDKLLLSSNDKVVLHMG
 731 DDMPANSYKGVLTSVRYGKGEMTADEQGNSETRTQGIGVIISNNPNLKLDSNDQVVLNMG
1079          DILTSVRFGDGIMNASDKGSTTARTQGIGVIVSNNDALAL KGDTVTLHMG
 341          DVLTSVRFGKGAMTANDLGDAETRTEGVGLIISNNPKLQLGQQDNVVLHMG
 398          GILTSVRFGKGAMNATDTGTDETRTEGIGVVISNNTNLKLNDGESVVLHMG

1515 AAHKNQKYRAVILTTEDGVKNYTNDTDAPVAYTDANGDLHFTNTNLDG     QQYTAVRG
1310 AAHKNQKFKAVLLTTNDGIQSF NDDNAPVAYTDANGDLVLSGKDITTDGVIQHNTAVKG
 791 AAHENQTYRPVLLTTKDGLKNYDSDSSVPQNALVSTNDKGQLIFKASS          IQG
1129 IAHANQAYRALLLTTTDGLMKYTSDNGAPIRYTDANGDLIFTSADI            KG
 392 LAHANQAFRAVVLTTATGLTIY NDDDAPIRYTDNKGDLIFTNHDV            YG
 449 AAHKNQKYRAVILTTEDGVKNYTNDTDAPVAYTDANGDLHFTNTNLDG     QQYTAVRG
```

```
1571 YANPDVTGYLAVWVPAGAADDQDARTAPSDEAHTTKTAYRSNAALDSNVIYEGFSNFIYW
1369 YANADVKGYLAVWVPVGASVQQDIRTAPSGVQSDGKSVYHSNAALDSNIIFEGFSNFVYW
 842 VSNPQVSGYLSVWVPVGAKDNQDARTASSSQPSTDGKTYHSNAALDSQVIYEGFSNFQSI
1177 YQNVEVSGFLSVWVPVGASDTQDARATGSSAANKTGDTLHSNAALDSNVIYEGFSNFQEM
 439 VLNPQVSGFLAMWVPTGAPANQDARSTASTNMSTDGSAYHSNAALDSQVIFESFSNFQAM
 505 YANPDVTGYLAVWVPAGAADD

1631 PTTESERTNVRIAQNADLFKSWGITTFELAPQYNSSKDGTFLDSIIDNGYAFTDRYDLGM
1429 PTNNSERANVKIAQNTDLFKELGITSFELAPQYNSSKDGTFLDSQIDNGYAFTDRYDLGM
 902 PTNTEDFTNVKIAQNANLFKSLGITSFELAPQYRSSNDNSFLDSVVQNGYAFTDRYDIGY
1237 PTAHDEFTNVKIAQNADLFKSWGVTSFQLAPQYRSSDDTSFLDSIIKNGYAFTDRYDLGF
 499 PTSHDTYTNVVLANHADQLHDWGITSVQLAPQYRSSTDGTFLDAIIQNGYAFTDRYDLGF

1691 STPNKYGSDEDLRNALQALHKAGLQAIADWVPDQIYNLPGKEAVTVTRSDDHGTTWEVSP
1489 SIPNKYGSDTDLRNAIKALHKAGIQAMADWVPDQIYNLPGKEVVTATRVDERGNDWNVAQ
 962 NTPTKYGTVTQLLDALRALHANGIQAIDDWVPDQIYNLPGEEIVAAQRTNGSGTYDQDSV
1297 NTPTKYGDVDDLADAIRAMHSVGIQVMADFVPDQIYNLPGQEVVAVNRTNNFGTPNQDSD
 559 GTPTKYGDDTDLRNVIKALHANGMQVMADFVPDQLYTLPGKELVQVTRTNNMGEPDTHSD
```

SEQUENCE LISTING

<110> TNO

<120> Novel glucans and novel glucansucrases derived from lactic acid bacteria

<130> Novel glucans and glucansucrases

<140>
<141>

<160> 10

<170> PatentIn Ver. 2.1

<210> 1
<211> 665
<212> DNA
<213> Lactobacillus reuteri

<400> 1
gataatacga atccggtggt gcaagctgaa gaattaaact ggctttacta tttaatgaat 60
ttcggtacaa ttacaggaaa taatcctgaa gctaattttg atggtattcg agtggatgct 120
gttgataatg tagatgttga cttattgagt attgcacgtg attactttaa tgcagcatat 180
aacatggagc aaagtgatgc cagtgctaat aagcacatta atattttgga agattgggga 240
tgggatgatc ctgcttatgt aaataagatt ggaaatcctc aattaacaat ggatgatcgt 300
ttacgaaatg caattatgga tacattatca ggagcacctg ataaaaacca agcattgaat 360
aaattaatta ctcagtcatt agtaaatcgt gctaatgata atactgaaaa cgcggttatt 420
ccaagctata attttgttcg agcacatgat agtaatgctc aagaccaaat tcgtcaggct 480
attcaagctg caactggaaa accatatggc gaatttaact tagatgatga aaagaagggt 540
atggaagcat atattaatga tcagaattct actaataaga agtggaatct ttacaatatg 600
ccttctgctt atactattct tctaacaaat aaagattcag ttccccacgt ctactatggc 660
gacat 665

<210> 2
<211> 221
<212> PRT
<213> Lactobacillus reuteri

<400> 2
Asp Asn Thr Asn Pro Val Val Gln Ala Glu Glu Leu Asn Trp Leu Tyr
1 5 10 15

Tyr Leu Met Asn Phe Gly Thr Ile Thr Gly Asn Asn Pro Glu Ala Asn
20 25 30

Phe Asp Gly Ile Arg Val Asp Ala Val Asp Asn Val Asp Val Asp Leu
35 40 45

Leu Ser Ile Ala Arg Asp Tyr Phe Asn Ala Ala Tyr Asn Met Glu Gln
50 55 60

Ser Asp Ala Ser Ala Asn Lys His Ile Asn Ile Leu Glu Asp Trp Gly
65 70 75 80

Trp Asp Asp Pro Ala Tyr Val Asn Lys Ile Gly Asn Pro Gln Leu Thr
85 90 95

Met Asp Asp Arg Leu Arg Asn Ala Ile Met Asp Thr Leu Ser Gly Ala

```
          100                 105                 110

Pro Asp Lys Asn Gln Ala Leu Asn Lys Leu Ile Thr Gln Ser Leu Val
        115                 120                 125

Asn Arg Ala Asn Asp Asn Thr Glu Asn Ala Val Ile Pro Ser Tyr Asn
    130                 135                 140

Phe Val Arg Ala His Asp Ser Asn Ala Gln Asp Gln Ile Arg Gln Ala
145                 150                 155                 160

Ile Gln Ala Ala Thr Gly Lys Pro Tyr Gly Glu Phe Asn Leu Asp Asp
                165                 170                 175

Glu Lys Lys Gly Met Glu Ala Tyr Ile Asn Asp Gln Asn Ser Thr Asn
            180                 185                 190

Lys Lys Trp Asn Leu Tyr Asn Met Pro Ser Ala Tyr Thr Ile Leu Leu
        195                 200                 205

Thr Asn Lys Asp Ser Val Pro His Val Tyr Tyr Gly Asp
    210                 215                 220
```

<210> 3
<211> 674
<212> DNA
<213> Lactobacillus strain LB 33

<400> 3
gacaattcga atccggtggt gcaagcggaa cagttaaact ggttatacta catgatgaat 60
ataggtagca ttactgccaa tgatcccacc gcaaactttg atggctatcg agtggacgct 120
gtggacaatg tcgatgctga tttattaaat atagctgccg attatgccaa agatgcttat 180
aaaactaatc aaagtgatgc taatgccaac aaacatttat caatattaga agattgggat 240
aataatgatc cggcttatat caaagcacat ggaaataatc agttaactat ggatttccca 300
gcacatttag caattaaata ttcattaaat atgccagtaa gtcaacgaag tgggctggaa 360
ccagagctca caaccagttt agttaacaga actggtgatg attctactga aaatgtcgca 420
cagccaaact atacttttat tagggctcac gatagtgaag tgcaaacaat catcgcacaa 480
attatcaaag ataaaatcaa ccctaactct gacggattaa cagttactcc cgatgaaata 540
agtcaggcct ttaaaatata taatgcagat gaattaaaga ctgataaaca atatactttt 600
tataacatgc cctctgccta tactattttg ctaaccaata aagatacagt accccacctc 660
tattacggcg acat 674

<210> 4
<211> 224
<212> PRT
<213> Lactobacillus strain LB 33

<400> 4
```
Asp Asn Ser Asn Pro Val Val Gln Ala Glu Gln Leu Asn Trp Leu Tyr
  1               5                  10                  15

Tyr Met Met Asn Ile Gly Ser Ile Thr Ala Asn Asp Pro Thr Ala Asn
            20                  25                  30

Phe Asp Gly Tyr Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu
        35                  40                  45

Leu Asn Ile Ala Ala Asp Tyr Ala Lys Asp Ala Tyr Lys Thr Asn Gln
```

50 55 60

Ser Asp Ala Asn Ala Asn Lys His Leu Ser Ile Leu Glu Asp Trp Asp
65 70 75 80

Asn Asn Asp Pro Ala Tyr Ile Lys Ala His Gly Asn Asn Gln Leu Thr
85 90 95

Met Asp Phe Pro Ala His Leu Ala Ile Lys Tyr Ser Leu Asn Met Pro
100 105 110

Val Ser Gln Arg Ser Gly Leu Glu Pro Glu Leu Thr Thr Ser Leu Val
115 120 125

Asn Arg Thr Gly Asp Asp Ser Thr Glu Asn Val Ala Gln Pro Asn Tyr
130 135 140

Thr Phe Ile Arg Ala His Asp Ser Glu Val Gln Thr Ile Ile Ala Gln
145 150 155 160

Ile Ile Lys Asp Lys Ile Asn Pro Asn Ser Asp Gly Leu Thr Val Thr
165 170 175

Pro Asp Glu Ile Ser Gln Ala Phe Lys Ile Tyr Asn Ala Asp Glu Leu
180 185 190

Lys Thr Asp Lys Gln Tyr Thr Phe Tyr Asn Met Pro Ser Ala Tyr Thr
195 200 205

Ile Leu Leu Thr Asn Lys Asp Thr Val Pro His Leu Tyr Tyr Gly Asp
210 215 220

<210> 5
<211> 671
<212> DNA
<213> Leuconostoc strain 86

<400> 5
gacaacacga acccggtggt gcaagctgag cagttaaatt ggttacacta tttgatgaat 60
tttggtagca ttacagcaaa cgattctgct gctaattttg atgagatacg tgtcgatgct 120
gtcgataatg ttgacgctga tttactccag attgcagcag attatttcaa agctgcttat 180
ggtgttgata aaaatgacgc aacagcaaat caacatcttt caattcttga agattggagc 240
cataacgacc ctgaatacgt gaaggatttt ggtaataatc aactcacaat ggatgattac 300
atgcataccc agttaatctg gtcgttgact aaagatatgc gtatgcgtgg taccatgcaa 360
cgcttcatgg actattacct cgtcaatcgc aatcacgata gtaccgaaaa cactgccatt 420
ccaaattaca gctttgttcg cgcacacgat agtgaagtac aaacagtcat tgctcaaatt 480
atttctgagt tacatcccga cgtaaaaaat agtttggcac caacagcaga ccagctagcc 540
gaagccttta aagtttataa taacgatgaa aaacaggcgg ataagaaata tacacaatac 600
aacatgccta gcgcctatgc gatgctgtta actaataaag atacagtacc ccgcgtctac 660
tatggcgaca t 671

<210> 6
<211> 223
<212> PRT
<213> Leuconostoc strain 86

<400> 6
Asp Asn Thr Asn Pro Val Val Gln Ala Glu Gln Leu Asn Trp Leu His
1 5 10 15

Tyr Leu Met Asn Phe Gly Ser Ile Thr Ala Asn Asp Ser Ala Ala Asn
20 25 30

Phe Asp Glu Ile Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu
35 40 45

Leu Gln Ile Ala Ala Asp Tyr Phe Lys Ala Ala Tyr Gly Val Asp Lys
50 55 60

Asn Asp Ala Thr Ala Asn Gln His Leu Ser Ile Leu Glu Asp Trp Ser
65 70 75 80

His Asn Asp Pro Glu Tyr Val Lys Asp Phe Gly Asn Asn Gln Leu Thr
85 90 95

Met Asp Asp Tyr Met His Thr Gln Leu Ile Trp Ser Leu Thr Lys Asp
100 105 110

Met Arg Met Arg Gly Thr Met Gln Arg Phe Met Asp Tyr Tyr Leu Val
115 120 125

Asn Arg Asn His Asp Ser Thr Glu Asn Thr Ala Ile Pro Asn Tyr Ser
130 135 140

Phe Val Arg Ala His Asp Ser Glu Val Gln Thr Val Ile Ala Gln Ile
145 150 155 160

Ile Ser Glu Leu His Pro Asp Val Lys Asn Ser Leu Ala Pro Thr Ala
165 170 175

Asp Gln Leu Ala Glu Ala Phe Lys Val Tyr Asn Asn Asp Glu Lys Gln
180 185 190

Ala Asp Lys Lys Tyr Thr Gln Tyr Asn Met Pro Ser Ala Tyr Ala Met
195 200 205

Leu Leu Thr Asn Lys Asp Thr Val Pro Arg Val Tyr Tyr Gly Asp
210 215 220

<210> 7
<211> 746
<212> DNA
<213> Leuconostoc strain 86

<400> 7
gactcactat agggcgaatt gggccctcta gatgcatgct cgagcggccg ccagtgtgat 60
ggatatctgc agaattcgcc cttgacaatt ggaatccggt ggtgcaagct gaacagttga 120
attggctaca ctatttgatg aatcttggaa cgattacagc taatgatcca gatgctaatt 180
ttgacagcat aagagttgac gctgtcgata atgtggatgc agatttgtta gatattgcac 240
gtgattactt taatgcagta tacaaggtta accaaagtga tgttaatgct aataaacata 300
tttctatttt agaagattgg agtggattag atcccaatga ggttgttaaa aatgggaatc 360
cacaattaac acttaacaca ggggttcaaa attcattatt aaatgctttg acaaaggggc 420
caaataatcg ttgggggggat agactcattg attgataaat caacaatgag atatccagat 480
aaggatggta aaatccttat tcctaattat agtttcgtac gtgcacacga tagtgaagtt 540

caaggtatta ttggcaaata ttaacagatc atacgtcagc cgaatcaggt aataaattca 600
caaaggatcc attaaaacag gcttggatta ttactatgct gaatcaagaw tagactgtta 660
aagaatattc gcactataat atggcgagtg cttatgcagc attgttaaca aattaagata 720
ccattcccaa ctctactacg gcgact 746

<210> 8
<211> 221
<212> PRT
<213> Leuconostoc strain 86

<400> 8
Asp Asn Trp Asn Pro Val Val Gln Ala Glu Gln Leu Asn Trp Leu His
1 5 10 15

Tyr Leu Met Asn Leu Gly Thr Ile Thr Ala Asn Asp Pro Asp Ala Asn
20 25 30

Phe Asp Ser Ile Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu
35 40 45

Leu Asp Ile Ala Arg Asp Tyr Phe Asn Ala Val Tyr Lys Val Asn Gln
50 55 60

Ser Asp Val Asn Ala Asn Lys His Ile Ser Ile Leu Glu Asp Trp Ser
65 70 75 80

Gly Leu Asp Pro Asn Glu Val Val Lys Asn Gly Asn Pro Gln Leu Thr
85 90 95

Leu Asn Thr Gly Val Gln Asn Ser Leu Leu Asn Ala Leu Thr Lys Gly
100 105 110

Pro Asn Asn Arg Trp Gly Ile Asp Ser Leu Ile Asp Lys Ser Thr Met
115 120 125

Arg Tyr Pro Asp Lys Asp Gly Lys Ile Leu Ile Pro Asn Tyr Ser Phe
130 135 140

Val Arg Ala His Asp Ser Glu Val Gln Gly Ile Ile Gly Lys Ile Leu
145 150 155 160

Thr Asp His Thr Ser Ala Glu Ser Gly Asn Lys Phe Thr Lys Asp Gln
165 170 175

Leu Lys Gln Ala Leu Asp Tyr Tyr Tyr Ala Asp Gln Asp Lys Thr Val
180 185 190

Lys Glu Tyr Ser His Tyr Asn Met Ala Ser Ala Tyr Ala Ala Leu Leu
195 200 205

Thr Asn Lys Asn Thr Ile Pro Asn Leu Tyr Tyr Gly Asp
210 215 220

<210> 9
<211> 670
<212> DNA
<213> Leuconostoc strain 86

<400> 9
gataattcga atccgatggt gcaagctgag caattgaact ggcttcacta catgatgaac 60
attggtacta tagctcagaa cgacccaaca gctaattttg acggttatcg tgttgatgcg 120
gttgataatg ttgatgccga tctcttacaa attgctggtg attactttaa agctgcatac 180
ggtactggta aaactgaggc aaacgcaaac aatcatattt cgatcttgga agattgggat 240
aataatgatt ctgcgtacat taaagcccac gggaacaacc aattgacaat ggattttcca 300
gcacacttgg ctttgaaata cgccttgaac atgcctcttg ccgcacaaag tggcctagaa 360
ccgctaatta atacaagtct tgttaagcgt gggaaagatg ccacagaaaa tgaagcacaa 420
ccaaactatg cctttatccg tgcccatgat agtgaagtgc agacagttat tgcacaaatt 480
attaaggata aaattaacac aaaatcagac ggcttaactg taacaccaga tgagattaag 540
caagctttca atatttacaa cgccgatgaa ttaaaagcag ataaggaata tacagcatac 600
aatattcctg cttcttacgc tgtattgttg acaaacaagg atactgtgcc cgcgtctact 660
atggcgacct 670

<210> 10
<211> 223
<212> PRT
<213> Leuconostoc strain 86

<400> 10
Asp Asn Ser Asn Pro Met Val Gln Ala Glu Gln Leu Asn Trp Leu His
1 5 10 15

Tyr Met Met Asn Ile Gly Thr Ile Ala Gln Asn Asp Pro Thr Ala Asn
20 25 30

Phe Asp Gly Tyr Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu
35 40 45

Leu Gln Ile Ala Gly Asp Tyr Phe Lys Ala Ala Tyr Gly Thr Gly Lys
50 55 60

Thr Glu Ala Asn Ala Asn Asn His Ile Ser Ile Leu Glu Asp Trp Asp
65 70 75 80

Asn Asn Asp Ser Ala Tyr Ile Lys Ala His Gly Asn Asn Gln Leu Thr
85 90 95

Met Asp Phe Pro Ala His Leu Ala Leu Lys Tyr Ala Leu Asn Met Pro
100 105 110

Leu Ala Ala Gln Ser Gly Leu Glu Pro Leu Ile Asn Thr Ser Leu Val
115 120 125

Lys Arg Gly Lys Asp Ala Thr Glu Asn Glu Ala Gln Pro Asn Tyr Ala
130 135 140

Phe Ile Arg Ala His Asp Ser Glu Val Gln Thr Val Ile Ala Gln Ile
145 150 155 160

Ile Lys Asp Lys Ile Asn Thr Lys Ser Asp Gly Leu Thr Val Thr Pro
165 170 175

Asp Glu Ile Lys Gln Ala Phe Asn Ile Tyr Asn Ala Asp Glu Leu Lys
180 185 190

Ala Asp Lys Glu Tyr Thr Ala Tyr Asn Ile Pro Ala Ser Tyr Ala Val
195 200 205

Leu Leu Thr Asn Lys Asp Thr Val Pro Ala Ser Thr Met Ala Thr

210 215 220

SEQ ID No. 11 DNA
SEQ ID No. 12 PRT
Lactobacillus reuteri strain 180

```
1     GAATTCGCTACCGAGATCTTAAAGGATAAAGACGTTGAAGTCGCTCCCGTTGATTCTTCA
1       N  S  L  P  R  S  *  R  I  K  T  L  K  S  L  P  L  I  L  Q

61    GCATATCCAGCCAAAGCTTACCGTCCTCGCCATTCAGTTATGAGTTTGAAGAAGGCAGAA
21      H  I  Q  P  K  L  T  V  L  A  I  Q  L  *  V  *  R  R  Q  K

121   GACACTGGGTTTGAGATTATGGATTGGCGGACTGCATTGAGTAAGTTTATAGAGGGGATT
41      T  L  G  L  R  L  W  I  G  G  L  H  *  V  S  L  *  R  G  L

181   GAGGAGTAAGATACTGGAACCGGTTTGGATTGGATACTGCTTTTTTATGGGCGGCGCAAT
61      R  S  K  I  L  E  P  V  W  I  G  Y  C  F  F  M  G  G  A  I

241   AAAGCTAGATCTAACTGGAAAAGACTGCGAACAAAATTGAAATTTAGTGTAAGCAGCTAA
81      K  L  D  L  T  G  K  D  C  E  Q  N  *  N  L  V  *  A  A  N

301   TATCCTTAGTCAATGTAGTATAATTGCAAATTTTTTACTAGGTAAGAAAGTATATTGTGG
101     I  L  S  Q  C  S  I  I  A  N  F  L  L  G  K  K  V  Y  C  G

361   AAATATTTAAGAATATTGTCGTTACCGGTAGAGACAATTTTATAAGTTCTAACTTTGTTC
121     N  I  *  E  Y  C  R  Y  R  *  R  Q  F  Y  K  F  *  L  C  S

421   ACTATGTTGTTAACCCTTACTAGGAAGTTGAACATATTACGGTTTTAGATAAGTTAACTT
141     L  C  C  *  P  L  L  G  S  *  T  Y  Y  G  F  R  *  V  N  L

481   ATACTGGCATTTAGTCAATTCTGATATCTTTGTTTAAAATTACAAATTTGAACTTTGTTT
161     Y  W  H  L  V  N  S  D  I  F  V  *  N  Y  K  F  E  L  C  L
                                  - 35
541   GAAGAAAATGTGGGAAGAATTTGAAAATTTCCTTTAAAAAAATTAAACATCATAGTATTA
181     K  K  M  W  E  E  F  E  N  F  L  *  K  N  *  T  S  *  Y  Y
        -10                                    RBS       start
601   TAATATCGATAATTAAATTGTTTATTCTGACATGAAGGAGATTAAAATGGAAATAAAGAA
201     N  I  D  N  *  I  V  Y  S  D  M  K  E  I  K  M  E  I  K  K

661   ACATTTTAAGTTGTATAAAAGTGGTAAACAATGGGTGACAGCGGCAGTTGCTACTGTTGC
221     H  F  K  L  Y  K  S  G  K  Q  W  V  T  A  A  V  A  T  V  A

721   CGTTTCAACCGCGCTTCTTTACGGGGGAGTTGCGCATGCTGATCAACAAGTTCAGTCTTC
241     V  S  T  A  L  L  Y  G  G  V  A  H  A  D  Q  Q  V  Q  S  S

781   CACAACCCAAGAACAAACTTCTACTGTGAATGCTGATACTACTAAAACAGTAAATTTAGA
261     T  T  Q  E  Q  T  S  T  V  N  A  D  T  T  K  T  V  N  L  D

841   TACTAATACTGACCAACCAGCCCAAACAACTGATAAAAATCAAGTAGCAAATGACACTAC
281     T  N  T  D  Q  P  A  Q  T  T  D  K  N  Q  V  A  N  D  T  T

901   TACTAACCAAAGTAAAACTGATAGTACATCAACAACTGTTAAGAATCCTACTTTTATACC
301     T  N  Q  S  K  T  D  S  T  S  T  T  V  K  N  P  T  F  I  P

961   AGTTTCTACTTTGTCTTCATCAGATAATGAAAAACAAAGTCAAAATTATAATAAGCCGGA
321     V  S  T  L  S  S  S  D  N  E  K  Q  S  Q  N  Y  N  K  P  D

1021  TAATGGAAACTATGGAAATGTTGATGCAGCTTACTTTAATAATAATCAATTGCATATTTC
341     N  G  N  Y  G  N  V  D  A  A  Y  F  N  N  N  Q  L  H  I  S

1081  AGGATGGCACGCAACAAATGCATCTCAAGGAACAGATAGTCGTCAGGTGATTGTACGTGA
361     G  W  H  A  T  N  A  S  Q  G  T  D  S  R  Q  V  I  V  R  D
```

```
1141  TATCACAACTAAAACTGAATTAGGACGTACTAATGTAACAAACAATGTTTTACGCCCAGA
381     I  T  T  K  T  E  L  G  R  T  N  V  T  N  N  V  L  R  P  D

1201  TGTTAAAAATGTCCACAATGTTTATAACGCTGATAATTCTGGATTCGATGTCAACATCAA
401     V  K  N  V  H  N  V  Y  N  A  D  N  S  G  F  D  V  N  I  N

1261  CATTGACTTTAGTAAGATGAAGGACTATCGTGATTCAATTGAAATTGTTAGTCGATACAG
421     I  D  F  S  K  M  K  D  Y  R  D  S  I  E  I  V  S  R  Y  S

1321  TGGAAATGGTAAATCTGTTGATTGGTGGTCTCAACCGATTACCTTTGACAAAAATAATTA
441     G  N  G  K  S  V  D  W  W  S  Q  P  I  T  F  D  K  N  N  Y

1381  CGCATACCTTGACACATTTGAAGTTAAAAATGGGGAATTGCATGCAACAGGATGGAATGC
461     A  Y  L  D  T  F  E  V  K  N  G  E  L  H  A  T  G  W  N  A

1441  TACTAATAAGGCAATTAACTATAACCACCATTTTGTAATTTTATTTGATCGAACAAATGG
481     T  N  K  A  I  N  Y  N  H  H  F  V  I  L  F  D  R  T  N  G

1501  TAAAGAAGTGACTCGTCAAGAAGTTCGTGATGGTCAATCGCGTCCAGATGTTGCTAAGGT
501     K  E  V  T  R  Q  E  V  R  D  G  Q  S  R  P  D  V  A  K  V

1561  ATATCCACAAGTAGTTGGGGCAAATAACTCTGGCTTTGACGTGACATTTAATATTGGTGA
521     Y  P  Q  V  V  G  A  N  N  S  G  F  D  V  T  F  N  I  G  D

1621  TCTAGATTACACTCATCAATACCAAATTCTTAGTCGTTACAGCAATGCAGATAATGGCGA
541     L  D  Y  T  H  Q  Y  Q  I  L  S  R  Y  S  N  A  D  N  G  E

1681  AGGTGATTATGTTACTTACTGGTTTGCTCCACAATCAATTGCTCCTGCTAACCAAAGTAA
561     G  D  Y  V  T  Y  W  F  A  P  Q  S  I  A  P  A  N  Q  S  N

1741  TCAGGGTTATTTAGATTCATTTGATATTAGTAAAAATGGTGAAGTGACAGTAACTGGTTG
581     Q  G  Y  L  D  S  F  D  I  S  K  N  G  E  V  T  V  T  G  W

1801  GAATGCTACTGATCTATCTGAATTACAAACTAACCATTATGTAATTTTATTTGACCAAAC
601     N  A  T  D  L  S  E  L  Q  T  N  H  Y  V  I  L  F  D  Q  T

1861  CGCTGGTCAACAAGTTGCATCTGCAAAAGTTGATCTAATTTCCCGTCCAGATGTTGCGAA
621     A  G  Q  Q  V  A  S  A  K  V  D  L  I  S  R  P  D  V  A  K

1921  AGCTTACCCAACAGTAAAAACTGCTGAAACTTCTGGCTTTAAGGTAACATTTAAGGTTAG
641     A  Y  P  T  V  K  T  A  E  T  S  G  F  K  V  T  F  K  V  S

1981  TAATTTACAACCAGGTCATCAATATAGTGTCGTAAGCCGTTTTTCTGCCGATGAAAACGG
661     N  L  Q  P  G  H  Q  Y  S  V  V  S  R  F  S  A  D  E  N  G

2041  TAATGGTAATGATAAACGTCATACCGATTACTGGTACAGCCCAGTAACCTTAAATCAAAC
681     N  G  N  D  K  R  H  T  D  Y  W  Y  S  P  V  T  L  N  Q  T

2101  TGCTTCAAATATTGATACTATCACAATGACATCGAATGGATTGCATATTACTGGTTGGAT
701     A  S  N  I  D  T  I  T  M  T  S  N  G  L  H  I  T  G  W  M

2161  GGCAAGTGATAATTCAATTAATGAAGCAACTCCATATGCCATTATTCTTAATAATGGTAG
721     A  S  D  N  S  I  N  E  A  T  P  Y  A  I  I  L  N  N  G  R

2221  AGAGGTTACTCGTCAAAAATTAACTTTAATTGCGCGTCCAGATGTAGCAGCAGTATATCC
741     E  V  T  R  Q  K  L  T  L  I  A  R  P  D  V  A  A  V  Y  P

2281  TTCACTCTATAACAGTGCTGTTAGTGGATTTGATACTACCATTAAGTTGACTAATGCTCA
761     S  L  Y  N  S  A  V  S  G  F  D  T  T  I  K  L  T  N  A  Q

2341  ATACCAGGCGCTTAATGGTCAACTACAAGTATTGTTACGTTTTTCTAAAGCTGTTGATGG
781     Y  Q  A  L  N  G  Q  L  Q  V  L  L  R  F  S  K  A  V  D  G
```

```
2401  TAATCCAAACGGCACTAATACTGTAACAGATCAATTTAGTAAGAATTATGCAACTACTGG
801     N  P  N  G  T  N  T  V  T  D  Q  F  S  K  N  Y  A  T  T  G

2461  TGGAAACTTTGATTATGTCAAAGTAAACGGCAATCAAATTGAATTTAGTGGCTGGCATGC
821     G  N  F  D  Y  V  K  V  N  G  N  Q  I  E  F  S  G  W  H  A

2521  AACTAATCAATCAAATGATAAAAATTCTCAATGGATTATTGTTTTAGTTAATGGTAAAGA
841     T  N  Q  S  N  D  K  N  S  Q  W  I  I  V  L  V  N  G  K  E

2581  GGTAAAACGGCAATTAGTTAATGATACTAAGGATGGTGCTGCTGGGTTCAACCGTAATGA
861     V  K  R  Q  L  V  N  D  T  K  D  G  A  A  G  F  N  R  N  D

2641  TGTTTACAAAGTAAATCCGGCTATTGAAAATAGTATTATGTCTGGGTTCCAAGGTATTAT
881     V  Y  K  V  N  P  A  I  E  N  S  I  M  S  G  F  Q  G  I  I

2701  TACTTTACCTGTAACAGTTAAGGATGAAAATGTTCAGCTTGTTCATCGTTTTAGTAATGA
901     T  L  P  V  T  V  K  D  E  N  V  Q  L  V  H  R  F  S  N  D

2761  TGCAAAGACTGGTGAAGGTAATTATGTTGATTTCTGGTCAGAAGTAATGTCTGTTAAGGA
921     A  K  T  G  E  G  N  Y  V  D  F  W  S  E  V  M  S  V  K  D

2821  CAGCTTCCAAAAGGGTAATGGTCCGCTTAATCAATTTGGTTTACAAACTATTAACGGCCA
941     S  F  Q  K  G  N  G  P  L  N  Q  F  G  L  Q  T  I  N  G  Q

2881  ACAATATTATATTGACCCAACAACTGGCCAACCTCGTAAGAATTTCTTATTGCAAAATGG
961     Q  Y  Y  I  D  P  T  T  G  Q  P  R  K  N  F  L  L  Q  N  G

2941  GAACGATTGGATTTACTTTGACAAAGATACTGGTGCTGGAACTAATGCTCTTAAGTTACA
981     N  D  W  I  Y  F  D  K  D  T  G  A  G  T  N  A  L  K  L  Q

3001  ATTTGATAAGGGAACAATTTCTGCTGATGAGCAATATCGTCGAGGAAATGAAGCCTATAG
1001    F  D  K  G  T  I  S  A  D  E  Q  Y  R  R  G  N  E  A  Y  S

3061  TTATGATGACAAGAGTATTGAAAATGTAAATGGTTACTTAACAGCTGATACTTGGTACCG
1021    Y  D  D  K  S  I  E  N  V  N  G  Y  L  T  A  D  T  W  Y  R

3121  ACCAAAACAAATCTTAAAGGATGGTACTACTTGGACTGACTCTAAAGAAACAGATATGCG
1041    P  K  Q  I  L  K  D  G  T  T  W  T  D  S  K  E  T  D  M  R

3181  CCCAATTTTAATGGTATGGTGGCCAAATACTGTTACACAAGCATATTATCTTAACTACAT
1061    P  I  L  M  V  W  W  P  N  T  V  T  Q  A  Y  Y  L  N  Y  M

3241  GAAGCAATATGGTAATTTATTGCCGGCTAGTTTACCAAGCTTCAGTACAGATGCTGATTC
1081    K  Q  Y  G  N  L  L  P  A  S  L  P  S  F  S  T  D  A  D  S

3301  TGCTGAATTAAATCATTACTCCGAGCTTGTTCAACAAAATATCGAAAAGCGGATCAGTGA
1101    A  E  L  N  H  Y  S  E  L  V  Q  Q  N  I  E  K  R  I  S  E

3361  GACTGGTAGTACTGATTGGTTACGTACACTAATGCATGAGTTCGTTACTAAGAATTCTAT
1121    T  G  S  T  D  W  L  R  T  L  M  H  E  F  V  T  K  N  S  M

3421  GTGGAATAAGGATAGTGAAAATGTCGATTACGGTGGTTTGCAATTACAAGGTGGATTCCT
1141    W  N  K  D  S  E  N  V  D  Y  G  G  L  Q  L  Q  G  G  F  L

3481  TAAGTATGTAAATAGTGATCTTACTAAATATGCAAATTCAGATTGGCGTTTAATGAACCG
1161    K  Y  V  N  S  D  L  T  K  Y  A  N  S  D  W  R  L  M  N  R

3541  TACAGCTACTAATATTGATGGTAAGAACTATGGTGGTGCGGAATTCTTATTAGCTAATGA
1181    T  A  T  N  I  D  G  K  N  Y  G  G  A  E  F  L  L  A  N  D

3601  TATTGATAACTCAAATCCAGTTGTTCAAGCTGAAGAATTAAACTGGCTTTACTATTTAAT
1201    I  D  N  S  N  P  V  V  Q  A  E  E  L  N  W  L  Y  Y  L  M
```

```
3661  GAATTTCGGTACAATTACAGGAAATAATCCTGAAGCTAATTTTGATGGTATTCGAGTGGA
1221    N  F  G  T  I  T  G  N  N  P  E  A  N  F  D  G  I  R  V  D

3721  TGCTGTTGATAATGTAGATGTTGACTTATTGAGTATTGCACGTGATTACTTTAATGCAGC
1241    A  V  D  N  V  D  V  D  L  L  S  I  A  R  D  Y  F  N  A  A

3781  ATATAACATGGAGCAAAGTGATGCCAGTGCTAATAAGCACATTAATATTTTGGAAGATTG
1261    Y  N  M  E  Q  S  D  A  S  A  N  K  H  I  N  I  L  E  D  W

3841  GGGATGGGATGATCCTGCTTATGTAAATAAGATTGGAAATCCTCAATTAACAATGGATGA
1281    G  W  D  D  P  A  Y  V  N  K  I  G  N  P  Q  L  T  M  D  D

3901  TCGTTTACGAAATGCAATTATGGATACATTATCAGGAGCACCTGATAAAAACCAAGCATT
1301    R  L  R  N  A  I  M  D  T  L  S  G  A  P  D  K  N  Q  A  L

3961  GAATAAATTAATTACTCAGTCATTAGTAAATCGTGCTAATGATAATACTGAAAACGCGGT
1321    N  K  L  I  T  Q  S  L  V  N  R  A  N  D  N  T  E  N  A  V

4021  TATTCCAAGCTATAATTTTGTTCGAGCACATGATAGTAATGCTCAAGACCAAATTCGTCA
1341    I  P  S  Y  N  F  V  R  A  H  D  S  N  A  Q  D  Q  I  R  Q

4081  GGCTATTCAAGCTGCAACTGGAAAACCATATGGCGAATTTAACTTAGATGATGAAAAGAA
1361    A  I  Q  A  A  T  G  K  P  Y  G  E  F  N  L  D  D  E  K  K

4141  GGGTATGGAAGCATATATTAATGATCAGAATTCTACTAATAAGAAGTGGAATCTTTACAA
1381    G  M  E  A  Y  I  N  D  Q  N  S  T  N  K  K  W  N  L  Y  N

4201  TATGCCTTCTGCTTATACTATTCTTCTAACAAATAAAGATTCAGTTCCTCGTGTTTACTA
1401    M  P  S  A  Y  T  I  L  L  T  N  K  D  S  V  P  R  V  Y  Y

4261  TGGAGACCTCTACCAAGATGGTGGTCAATATATGGAACATAAAACACGTTACTTTGATAC
1421    G  D  L  Y  Q  D  G  G  Q  Y  M  E  H  K  T  R  Y  F  D  T

4321  TATTACTAACTTATTAAAGACACGGGTTAAATATGTTGCCGGTGGACAAACTATGAGTGT
1441    I  T  N  L  L  K  T  R  V  K  Y  V  A  G  G  Q  T  M  S  V

4381  TGATAAGAATGGTATTCTTACAAACGTTCGTTTTGGGAAAGGCGCCATGAATGCTACTGA
1461    D  K  N  G  I  L  T  N  V  R  F  G  K  G  A  M  N  A  T  D

4441  TACTGGTACTGATGAAACAAGAACAGAAGGTATCGGTGTTGTAATTAGTAACAATACTAA
1481    T  G  T  D  E  T  R  T  E  G  I  G  V  V  I  S  N  N  T  N

4501  TTTGAAGCTTAATGATGGTGAATCAGTAGTGCTTCATATGGGAGCTGCTCATAAGAATCA
1501    L  K  L  N  D  G  E  S  V  V  L  H  M  G  A  A  H  K  N  Q

4561  AAAGTATCGTGCTGTGATCTTAACAACTGAAGATGGTGTTAAGAATTACACTAATGATAC
1521    K  Y  R  A  V  I  L  T  T  E  D  G  V  K  N  Y  T  N  D  T

4621  AGACGCACCAGTTGCATACACTGATGCTAATGGTGACCTTCACTTTACTAATACTAATTT
1541    D  A  P  V  A  Y  T  D  A  N  G  D  L  H  F  T  N  T  N  L

4681  AGATGGTCAACAATATACAGCTGTTCGTGGATATGCAAATCCTGATGTAACAGGATATCT
1561    D  G  Q  Q  Y  T  A  V  R  G  Y  A  N  P  D  V  T  G  Y  L

4741  AGCTGTTTGGGTACCAGCTGGAGCAGCAGATGATCAAGATGCACGTACTGCACCAAGTGA
1581    A  V  W  V  P  A  G  A  A  D  D  Q  D  A  R  T  A  P  S  D

4801  TGAGGCCCATACTACAAAGACTGCTTATCGCTCTAATGCAGCCCTTGATTCTAACGTTAT
1601    E  A  H  T  T  K  T  A  Y  R  S  N  A  A  L  D  S  N  V  I

4861  TTATGAAGGATTCTCTAACTTCATTTACTGGCCAACTACTGAAAGCGAACGGACTAATGT
1621    Y  E  G  F  S  N  F  I  Y  W  P  T  T  E  S  E  R  T  N  V
```

```
4921  GAGAATTGCACAAAATGCGGATCTATTTAAGTCATGGGGAATTACTACCTTTGAATTAGC
1641    R  I  A  Q  N  A  D  L  F  K  S  W  G  I  T  T  F  E  L  A

4981  TCCACAATACAATTCAAGTAAAGATGGTACGTTCCTTGATTCAATAATTGATAATGGATA
1661    P  Q  Y  N  S  S  K  D  G  T  F  L  D  S  I  I  D  N  G  Y

5041  TGCCTTTACTGATCGTTATGATTTAGGAATGAGTACTCCTAACAAGTATGGATCTGATGA
1681    A  F  T  D  R  Y  D  L  G  M  S  T  P  N  K  Y  G  S  D  E

5101  AGACTTACGTAATGCTTTACAAGCCTTACATAAAGCTGGTTTACAAGCAATTGCCGACTG
1701    D  L  R  N  A  L  Q  A  L  H  K  A  G  L  Q  A  I  A  D  W

5161  GGTTCCTGATCAAATTTATAACTTACCTGGTAAAGAAGCTGTAACAGTAACACGTTCAGA
1721    V  P  D  Q  I  Y  N  L  P  G  K  E  A  V  T  V  T  R  S  D

5221  TGATCACGGTACTACATGGGAAGTTTCGCCAATAAAGAATGTTGTCTATATTACAAATAC
1741    D  H  G  T  T  W  E  V  S  P  I  K  N  V  V  Y  I  T  N  T

5281  GATTGGTGGAGGTGAATACCAGAAGAAATATGGTGGTGAATTCTTAGACACTCTTCAAAA
1761    I  G  G  G  E  Y  Q  K  K  Y  G  G  E  F  L  D  T  L  Q  K

5341  AGAATATCCACAATTATTTAGTCAGGTATATCCAGTAACTCAAACGACAATTGATCCTAG
1781    E  Y  P  Q  L  F  S  Q  V  Y  P  V  T  Q  T  T  I  D  P  S

5401  TGTTAAGATTAAAGAGTGGTCTGCTAAATACTTTAATGGTACTAATATCCTTCATCGAGG
1801    V  K  I  K  E  W  S  A  K  Y  F  N  G  T  N  I  L  H  R  G

5461  TGCTGGATATGTATTGCGCTCTAATGATGGTAAATACTATAATCTTGGTACAAGCACTCA
1821    A  G  Y  V  L  R  S  N  D  G  K  Y  Y  N  L  G  T  S  T  Q

5521  ACAATTCTTACCGTCTCAATTATCAGTTCAAGATAATGAAGGATATGGATTTGTAAAAGA
1841    Q  F  L  P  S  Q  L  S  V  Q  D  N  E  G  Y  G  F  V  K  E

5581  AGGAAATAATTACCATTACTATGATGAGAATAAACAGATGGTAAAAGATGCGTTTATTCA
1861    G  N  N  Y  H  Y  Y  D  E  N  K  Q  M  V  K  D  A  F  I  Q

5641  AGATAGTGTTGGTAATTGGTATTACTTCGATAAAAATGGTAATATGGTTGCTAACCAAAG
1881    D  S  V  G  N  W  Y  Y  F  D  K  N  G  N  M  V  A  N  Q  S

5701  TCCTGTTGAAATTAGTAGTAATGGAGCTTCAGGAACTTACCTTTTCTTGAACAATGGGAC
1901    P  V  E  I  S  S  N  G  A  S  G  T  Y  L  F  L  N  N  G  T

5761  ATCATTCCGTTCTGGATTGGTGAAAACTGATGCAGGTACGTACTATTATGATGGCGATGG
1921    S  F  R  S  G  L  V  K  T  D  A  G  T  Y  Y  Y  D  G  D  G

5821  CCGAATGGTTCGTAATCAAACGGTAAGTGATGGTGCGATGACATATGTTCTTGATGAAAA
1941    R  M  V  R  N  Q  T  V  S  D  G  A  M  T  Y  V  L  D  E  N

5881  TGGTAAACTTGTTAGTGAATCATTTGATTCATCTGCTACTGAAGCACACCCATTAAAACC
1961    G  K  L  V  S  E  S  F  D  S  S  A  T  E  A  H  P  L  K  P

5941  TGGTGATTTAAACGGCCAAAAATAATTACAATATGAAAATTGGAACTTGTATTTTACCTT
1981    G  D  L  N  G  Q  K  *  L  Q  Y  E  N  W  N  L  Y  F  T  F
                                inverted repeat
6001  CTTTGAAATAATATAGTTCTAATTAAGCAGCTCGCACCAAGACTTGGTATGAGCTGCTTT
2001    F  E  I  I  *  F  *  L  S  S  S  H  Q  D  L  V  *  A  A  F

6061  TTTTGGCTCTACAATATCTGGTGTTGATATAGAAATATCACTTTCTATACCAATATCAGA
2021    F  G  S  T  I  S  G  V  D  I  E  I  S  L  S  I  P  I  S  D

6121  TTTTTGTTTTTAAACTAAAAAAGAGGCTCGCCCTCTGATACAATGAAATCGCCAAATCAC
2041    F  C  F  *  T  K  K  E  A  R  P  L  I  Q  *  N  R  Q  I  T
```

```
6181  ATAGTAAAGAAGGTAACCTCCATGGATAATGATACAAGAACTCTTCTCAATTTAACAGAC
2061    *  *  R  R  *  P  P  W  I  M  I  Q  E  L  F  S  I  *  Q  T

6241  CCTCATTTAAATTTTCCTCATCATTGGCTTAAATATAAAACAATTAAAAAAGTTCGGGTG
2081    L  I  *  I  F  L  I  I  G  L  N  I  K  Q  L  K  K  F  G  W

6301  GCACAAATATNCTGTACCCTTTCTTATACACCACGGGNCTTGTCCAAATTGGGGGAGTCA
2101    H  K  Y  X  V  P  F  L  I  H  H  G  X  C  P  N  W  G  S  H

6361  TTAATCGNGGTCAAATCTTAAAATATGGGCTTTTATCAAGCTAAACACAATATGGACAAT
2121    *  S  X  S  N  L  K  I  W  A  F  I  K  L  N  T  I  W  T  I

6421  TTAAAACTCAACCATTAATGNTG
2141    *  N  S  T  I  N  X
```

SEQ ID No. 13 DNA
SEQ ID No. 14 PRT
Lactobacillus reuteri strain ML1

```
1     ATCGATAATCAAATTGTTTATTTTGATATAAAGGAGATTAAAATGGAAATAAAGAAACAT
1       I  D  N  Q  I  V  Y  F  D  I  K  E  I  K  M  E  I  K  K  H
                                      RBS         start
61    TTTAAGTTGTATAAAAGTGGTAAACAATGGGTGACAGCGGCTGTTGCTACTGTTGCCGTT
21      F  K  L  Y  K  S  G  K  Q  W  V  T  A  A  V  A  T  V  A  V

121   TCAACCGCGCTTCTTTACGGGGGAGTTGCACATGCTGATCAACAAGTTCAGTCTTCCACA
41      S  T  A  L  L  Y  G  G  V  A  H  A  D  Q  Q  V  Q  S  S  T

181   ACTCAAGACCAAACTTCTACTGTAAATACTAATACTACTAAAACAATAGCTGCAGATACT
61      T  Q  D  Q  T  S  T  V  N  T  N  T  T  K  T  I  A  A  D  T

241   AATGCTGATCAGCCAGCTCAAACAGCTGATAAAAATCAAGCAGCATCAAATGACACTACT
81      N  A  D  Q  P  A  Q  T  A  D  K  N  Q  A  A  S  N  D  T  T

301   AACCAAAGTAAAACTGATAGTACTTCAACAACTGTTAAGAATCTTACTTCTACACCAGTT
101     N  Q  S  K  T  D  S  T  S  T  T  V  K  N  L  T  S  T  P  V

361   TCTACTTTGCCATCAACTGATAATGAAAAACAAAATCAAAATTATAATAAGCATGATAAT
121     S  T  L  P  S  T  D  N  E  K  Q  N  Q  N  Y  N  K  H  D  N

421   GGAAACTATGGGAATATTGATACTGCTTACTTTAGCAATAATCAATTGCATGTTTCAGGA
141     G  N  Y  G  N  I  D  T  A  Y  F  S  N  N  Q  L  H  V  S  G

481   TGGAATGCAACGAATGCATCTCAAGGAACAAACAGTCGGCAAATTATTGTGCGTGATATC
161     W  N  A  T  N  A  S  Q  G  T  N  S  R  Q  I  I  V  R  D  I

541   ACAACCAATAATGAATTAGGTCGTACTGATGTAACAAACAATGTTGCGCGCCCAGACGTT
181     T  T  N  N  E  L  G  R  T  D  V  T  N  N  V  A  R  P  D  V

601   AAGAATGTTCATAATGTTTATAACGCTGATAATTCTGGATTTGATATTAATGTCAATATT
201     K  N  V  H  N  V  Y  N  A  D  N  S  G  F  D  I  N  V  N  I

661   GAATTTAGCAAGATGAAAGATTATCGGGATTCAATTGAAATTGTTAGTCGATACAGTGGA
221     E  F  S  K  M  K  D  Y  R  D  S  I  E  I  V  S  R  Y  S  G

721   AACGGTAAATCTATTGACTGGTGGTCCCAACCGATCACTTTTGACAAAAACAATTATGCT
241     N  G  K  S  I  D  W  W  S  Q  P  I  T  F  D  K  N  N  Y  A

781   TATCTTGATACATTTGAAGTGAAAAATGGCGAATTACATGCAACCGGATGGAATGCTACT
261     Y  L  D  T  F  E  V  K  N  G  E  L  H  A  T  G  W  N  A  T

841   AATAGTGCAATTAACTATAATCACCATTTTGTAATTTTATTTGATCAAACGAATGGTAAG
281     N  S  A  I  N  Y  N  H  H  F  V  I  L  F  D  Q  T  N  G  K
```

```
901   GAAGTAGCACGACAAGAAGTTCGTGAAGGCCAATCACGCCCAGATGTTGCTAAGGTATAT
301    E  V  A  R  Q  E  V  R  E  G  Q  S  R  P  D  V  A  K  V  Y

961   CCACAAGTAGTTGGTGCTGACAACTCCGGCTTTGATGTGACATTTAATATCGGTAATTTA
321    P  Q  V  V  G  A  D  N  S  G  F  D  V  T  F  N  I  G  N  L

1021  GATTATACTCACCAGTACCAAGTTCTTAGTCGTTACAGCAATTCTGATAATGGCGAAGGC
341    D  Y  T  H  Q  Y  Q  V  L  S  R  Y  S  N  S  D  N  G  E  G

1081  GATAATGTTACCTACTGGTTTAATCCACAATCCATTGCTCCTGCTAATCAAAGTAACCAG
361    D  N  V  T  Y  W  F  N  P  Q  S  I  A  P  A  N  Q  S  N  Q

1141  GGTTATCTAGACTCATTTGATATTAGTAAAAATGGTGAAGTAACAGTGACCGGATGGAAT
381    G  Y  L  D  S  F  D  I  S  K  N  G  E  V  T  V  T  G  W  N

1201  GCTACTGACTTGTCAGAATTACAAAATAACCATTATGTAATTCTATTTGATCAGACAGCA
401    A  T  D  L  S  E  L  Q  N  N  H  Y  V  I  L  F  D  Q  T  A

1261  GGCAAACAAGTAGCATCTGCCAAGGCTGATTTAATTTCACGTCCAGATGTTGCAAAGGCT
421    G  K  Q  V  A  S  A  K  A  D  L  I  S  R  P  D  V  A  K  A

1321  TATCCAACAGTAAAAACTGCTGCAAATTCTGGCTTTAAGGTAACATTTAAGGTTAATGAT
441    Y  P  T  V  K  T  A  A  N  S  G  F  K  V  T  F  K  V  N  D

1381  TTACAACCGGGTCACCAATATAGCGTTGTAAGTCGTTTCTCTGCCGATGAAAATGGTAAT
461    L  Q  P  G  H  Q  Y  S  V  V  S  R  F  S  A  D  E  N  G  N

1441  GGTAATGATAAGCGTCATACAGATTACTGGTTTAGTCCAGTAACATTAAACCAGAATGCT
481    G  N  D  K  R  H  T  D  Y  W  F  S  P  V  T  L  N  Q  N  A

1501  TCAAACATTGATACTATTACAATGACATCTAATGGGTTACATATTGGCAGTTGGATGGCA
501    S  N  I  D  T  I  T  M  T  S  N  G  L  H  I  G  S  W  M  A

1561  AGTGATAACTCAATTAATGAAACAACTCCATATGCTATTATTCTCAATAACGGTAAAGAA
521    S  D  N  S  I  N  E  T  T  P  Y  A  I  I  L  N  N  G  K  E

1621  GTTACTCGTCAAAAGATGAGTTTAACTGCCCGTCCAGATGTAGCAGCAGTATATCCTTCA
541    V  T  R  Q  K  M  S  L  T  A  R  P  D  V  A  A  V  Y  P  S

1681  CTTTATAATAGTGCTGTTAGTGGGTTTGATACTACTATTAAATTGACTAATGATCAGTAT
561    L  Y  N  S  A  V  S  G  F  D  T  T  I  K  L  T  N  D  Q  Y

1741  CAAGCGCTTAATGGTCAATTACAAGTATTGTTACGTTTTTCTAAAGCTGCTGATGGTAAT
581    Q  A  L  N  G  Q  L  Q  V  L  L  R  F  S  K  A  A  D  G  N

1801  CCAAGTGGTGATAATACTGTAACTGATCAATTTAGTAAAAATTATGCAACTACTGGTGGA
601    P  S  G  D  N  T  V  T  D  Q  F  S  K  N  Y  A  T  T  G  G

1861  AACTTTGATTATGTAAAAGTAAATGGTAATCAAGTTGAATTTAGTGGTTGGCATGCAACT
621    N  F  D  Y  V  K  V  N  G  N  Q  V  E  F  S  G  W  H  A  T

1921  AACCAATCAAATGATAAAGATTCACAATGGATTATTGTTTTAGTTAATGGTAAAGAAGTA
641    N  Q  S  N  D  K  D  S  Q  W  I  I  V  L  V  N  G  K  E  V

1981  AAGCGTCAATTAGTTAATGATACTAAAGAGGGGGCTGCTGGCTTCAACCGAAACGATGTC
661    K  R  Q  L  V  N  D  T  K  E  G  A  A  G  F  N  R  N  D  V

2041  TACAAAGTAAATCCAGCTATTGAAAACAGTTCTATGTCTGGATTCCAAGGCATTATTACT
681    Y  K  V  N  P  A  I  E  N  S  S  M  S  G  F  Q  G  I  I  T

2101  TTACCAGTAACAGTTAAGAATGAGAATGTTCAGATTGTCCATCGTTTTAGTAATGATGCA
701    L  P  V  T  V  K  N  E  N  V  Q  I  V  H  R  F  S  N  D  A
```

```
2161  AAGACAGGTGAAGGTAGCCATGTTGATTTCTGGTCAGAAGTAATGCCAGTTAAGGATAGT
721    K  T  G  E  G  S  H  V  D  F  W  S  E  V  M  P  V  K  D  S

2221  TTCCAAAAGGGTAATGGTCCGCTTAAGCAATTTGGCTTACAAACTATTAATGGTCATCAA
741    F  Q  K  G  N  G  P  L  K  Q  F  G  L  Q  T  I  N  G  H  Q

2281  TATTATATTGACCCAATGACTGGCCAACCTCGCAAGAACTTCCTATTACAAAATGGTAAT
761    Y  Y  I  D  P  M  T  G  Q  P  R  K  N  F  L  L  Q  N  G  N

2341  GACTGGCTTTATTTTGATAATGAAACTGGTGAGGGAACTAATGCGTTAAAGAGGCAATTT
781    D  W  L  Y  F  D  N  E  T  G  E  G  T  N  A  L  K  R  Q  F

2401  GACGGAGGAACGATTTCTGCTGATAGTCAGTATAGAAAGGGTAATGAAGCTTATGGTTAT
801    D  G  G  T  I  S  A  D  S  Q  Y  R  K  G  N  E  A  Y  G  Y

2461  GACAATAAGAGCATTGAAAATGTTGATGGCTTTTTAACAGCTGATACTTGGTACCGACCA
821    D  N  K  S  I  E  N  V  D  G  F  L  T  A  D  T  W  Y  R  P

2521  AAACAAATTTTAAAATGGACCACCTGGACAGATTCTAAAGAAACAGATATGCGACCGCTC
841    K  Q  I  L  K  W  T  T  W  T  D  S  K  E  T  D  M  R  P  L

2581  TTAATGGTTTGGTGGCCAAATACTGTAACCCAAGCATATTACCTTAACTACATGAAACAA
861    L  M  V  W  W  P  N  T  V  T  Q  A  Y  Y  L  N  Y  M  K  Q

2641  CATGGAAACTTATTACCAGCTAATCTTCCATTCTTTAATTCTGATGCAGATCCATTAGAA
881    H  G  N  L  L  P  A  N  L  P  F  F  N  S  D  A  D  P  L  E

2701  TTAAATTATTATGCAGAAATTGTTCAGCAAAATATTGAAAAGAAGATTAGTCAAACTGGT
901    L  N  Y  Y  A  E  I  V  Q  Q  N  I  E  K  K  I  S  Q  T  G

2761  AATACTGACTGGTTGCGAACTTTGATGCACGAATTTGTATCTAATAATACAATGTGGAAT
921    N  T  D  W  L  R  T  L  M  H  E  F  V  S  N  N  T  M  W  N

2821  AAGAATAGTGAAAATGAAGACTTTGGTGGGTTGCAATTACAAGGTGGTTTTCTAAAGTAC
941    K  N  S  E  N  E  D  F  G  G  L  Q  L  Q  G  G  F  L  K  Y

2881  GTTAATAGTGATAAGACACCTAATGCTAATTCTAATTGGCGTATTATGGGTAGGCAGCCA
961    V  N  S  D  K  T  P  N  A  N  S  N  W  R  I  M  G  R  Q  P

2941  GCTAATATTGACGGAAATGGGCCAATTGGATCAGAATTCTTATTAGCTAATGACGTTGAT
981    A  N  I  D  G  N  G  P  I  G  S  E  F  L  L  A  N  D  V  D

3001  AATTCTAATCCAGTTGTTCAAGCTGAACAGTTAAATTGGCTACATTACTTATTGAATTTT
1001   N  S  N  P  V  V  Q  A  E  Q  L  N  W  L  H  Y  L  L  N  F

3061  GGAACTATTACTGCAAATGATCCTGATGCTAATTTTGATAGCATTCGTGTTGATGCTGTT
1021   G  T  I  T  A  N  D  P  D  A  N  F  D  S  I  R  V  D  A  V

3121  GACAATGTAGATGCCGATTTATTAGATATAGCTGGTGATTACTTTAATGCAGTATATCAT
1041   D  N  V  D  A  D  L  L  D  I  A  G  D  Y  F  N  A  V  Y  H

3181  TCTCAAAGTAATGATAAAATTGCTAATGCTCATATTAATATTCTTGAGGATTGGGGTGGC
1061   S  Q  S  N  D  K  I  A  N  A  H  I  N  I  L  E  D  W  G  G

3241  CAAGATCCGTATTATACGCAAAGCATCGGAACTCCTCAATTATCGATGGATTATAATTTC
1081   Q  D  P  Y  Y  T  Q  S  I  G  T  P  Q  L  S  M  D  Y  N  F

3301  TCAACTATAAGAAGTGTGTTAGCATCTAACACTGCATCAATGACTGATATTATTAAGAAT
1101   S  T  I  R  S  V  L  A  S  N  T  A  S  M  T  D  I  I  K  N

3361  TCATTGGTAAATCGGAGCTTAGATAATGCTGAAAACGTATCAATTCCTAATTACTCATTT
1121   S  L  V  N  R  S  L  D  N  A  E  N  V  S  I  P  N  Y  S  F
```

```
3421  ATCCGTGCACATGATAATGGTTCACAAGATGATATTAAGCGTGCAATTTCAGATGTAAAT
1141   I  R  A  H  D  N  G  S  Q  D  D  I  K  R  A  I  S  D  V  N

3481  AATTTACCATATGGTTCGAAGTTTAACTTTGAGCAAGAGCAAAAGGGGATTGAAGCATAC
1161   N  L  P  Y  G  S  K  F  N  F  E  Q  E  Q  K  G  I  E  A  Y

3541  ATTGCAGATCAAAGTAATGTTAATAAGAAGTGGAATAATTATAATATTCCATCTTCATAT
1181   I  A  D  Q  S  N  V  N  K  K  W  N  N  Y  N  I  P  S  S  Y

3601  GCTATTATGTTGACTAATAAGGATACCGTTCCTCGTGTATATTATGGTGATTTATTTACT
1201   A  I  M  L  T  N  K  D  T  V  P  R  V  Y  Y  G  D  L  F  T

3661  GATGGTGGTCAGTATATGGCACAAACAACGCGTTATTATCCTGCACTTACAAGTCTTTTA
1221   D  G  G  Q  Y  M  A  Q  T  T  R  Y  Y  P  A  L  T  S  L  L

3721  AAGGCACGTATTAAGTATGTAGCTGGTGGACAAACAATGTCTGTCGATAAGAATAATATT
1241   K  A  R  I  K  Y  V  A  G  G  Q  T  M  S  V  D  K  N  N  I

3781  TTGACTAGTGTTCGCTTTGGTAAAGGTGCGATGAATCCTACTGATATGGGTGATAGTTTA
1261   L  T  S  V  R  F  G  K  G  A  M  N  P  T  D  M  G  D  S  L

3841  ACTAGAACATCTGGTGTTGGGGTAGTTATAAGTAATAATGATAAATTATTATTAAGCTCA
1281   T  R  T  S  G  V  G  V  V  I  S  N  N  D  K  L  L  L  S  S

3901  AATGATAAAGTTGTATTACACATGGGTGCTGCACATAAGAATCAGAAATTTAAAGCAGTC
1301   N  D  K  V  V  L  H  M  G  A  A  H  K  N  Q  K  F  K  A  V

3961  TTACTAACTACTAATGATGGTATTCAGAGTTTTAATGATGACAATGCGCCTGTTGCATAT
1321   L  L  T  T  N  D  G  I  Q  S  F  N  D  D  N  A  P  V  A  Y

4021  ACTGATGCTAATGGTGACTTGGTCCTTTCTGGTAAAGATATTACGACTGATGGTGTAATT
1341   T  D  A  N  G  D  L  V  L  S  G  K  D  I  T  T  D  G  V  I

4081  CAACATAATACTGCTGTTAAGGGCTATGCTAATGCTGATGTTAAAGGTTATCTTGCAGTA
1361   Q  H  N  T  A  V  K  G  Y  A  N  A  D  V  K  G  Y  L  A  V

4141  TGGGTTCCAGTAGGTGCCAGTGTACAACAGGATATTAGAACAGCACCATCAGGGGTACAA
1381   W  V  P  V  G  A  S  V  Q  Q  D  I  R  T  A  P  S  G  V  Q

4201  AGTGATGGAAAGTCTGTTTATCATTCAAATGCAGCTCTGGATTCAAATATTATTTTTGAA
1401   S  D  G  K  S  V  Y  H  S  N  A  A  L  D  S  N  I  I  F  E

4261  GGATTCTCTAACTTTGTATATTGGCCGACAAATAATTCTGAGCGTGCAAATGTAAAAATC
1421   G  F  S  N  F  V  Y  W  P  T  N  N  S  E  R  A  N  V  K  I

4321  GCTCAGAATACTGACTTATTTAAGGAGTTGGGTATTACTTCATTTGAATTAGCTCCACAG
1441   A  Q  N  T  D  L  F  K  E  L  G  I  T  S  F  E  L  A  P  Q

4381  TATAATTCAAGTAAGGATGGCACATTCCTTGATTCTCAGATTGATAATGGATATGCATTT
1461   Y  N  S  S  K  D  G  T  F  L  D  S  Q  I  D  N  G  Y  A  F

4441  ACTGATCGCTATGATCTAGGTATGAGCATTCCAAATAAGTATGGTAGCGATACTGATCTA
1481   T  D  R  Y  D  L  G  M  S  I  P  N  K  Y  G  S  D  T  D  L

4501  AGGAATGCTATTAAAGCCTTACATAAGGCCGGAATTCAAGCAATGGCTGATTGGGTTCCT
1501   R  N  A  I  K  A  L  H  K  A  G  I  Q  A  M  A  D  W  V  P

4561  GATCAAATTTATAATTTACCAGGTAAAGAAGTTGTTACTGCTACTCGTGTTGACGAACGT
1521   D  Q  I  Y  N  L  P  G  K  E  V  V  T  A  T  R  V  D  E  R

4621  GGAAATGATTGGAATGTAGCTCAGATTAAGGATTCACTTTATGTTGCTAATACAATTGGT
1541   G  N  D  W  N  V  A  Q  I  K  D  S  L  Y  V  A  N  T  I  G
```

```
4681  GGTGGAAAGTATCAAGAGCAATATGGTGGAGCTTTCCTTGATCAATTACAAAAGCAATAT
1561   G  G  K  Y  Q  E  Q  Y  G  G  A  F  L  D  Q  L  Q  K  Q  Y

4741  CCACAAATCTTTGAACGTAAACAACCTTCAACTGGTGTAGCAATTGACCCAAGTACTAAG
1581   P  Q  I  F  E  R  K  Q  P  S  T  G  V  A  I  D  P  S  T  K

4801  ATTAAACAGTGGTCTGCTAAATACTTTAATGGGACAAATATTTTACATCGTGGTGCAGGG
1601   I  K  Q  W  S  A  K  Y  F  N  G  T  N  I  L  H  R  G  A  G

4861  TATGTATTAAGAGATAACGGTGGTAACTACTTTAGCCTTGGAAATAGTAATAATAAACAG
1621   Y  V  L  R  D  N  G  G  N  Y  F  S  L  G  N  S  N  N  K  Q

4921  TTATTACCAAATCAATTATCAGGTAAGGCTGAAAATGGCTTTGTTGATGTTAATGGGAAT
1641   L  L  P  N  Q  L  S  G  K  A  E  N  G  F  V  D  V  N  G  N

4981  ACTAAATACTTTACATCAACCGGAATTCCTGTCACGGATGCATTTGTTCAAGACAGTGTA
1661   T  K  Y  F  T  S  T  G  I  P  V  T  D  A  F  V  Q  D  S  V

5041  GGTAACTGGTACTATATTGATAAAAATGGTAATATGCTTAAAAATACCGGTTTTGTAGAT
1681   G  N  W  Y  Y  I  D  K  N  G  N  M  L  K  N  T  G  F  V  D

5101  ATTACGCGAAATGGTCAGACAGGTACGTATCTATTCTTAAATAACGGTATCTCATTCCGA
1701   I  T  R  N  G  Q  T  G  T  Y  L  F  L  N  N  G  I  S  F  R

5161  TCAGGATTAGTTAAAATTGGTAATGATACTTATTACTTTGACGGTAATGGAAAAATGGTT
1721   S  G  L  V  K  I  G  N  D  T  Y  Y  F  D  G  N  G  K  M  V

5221  CGTGGCCAATCTATTAGTGATGGTACGATGAATTATACTCTTGATAAGGATGGTAAATTA
1741   R  G  Q  S  I  S  D  G  T  M  N  Y  T  L  D  K  D  G  K  L

5281  GTTGGCTTGTATTATGATCCAAGTAGTCAGAATCCACATCCAATTACTCAACAGGATTTA
1761   V  G  L  Y  Y  D  P  S  S  Q  N  P  H  P  I  T  Q  Q  D  L

5341  AGTGGTACTAATAAGTAGTTTATTAAAAATCACCAATAGAAGTTGTCTCTACATCAAATG
1781   S  G  T  N  K  *  F  I  K  N  H  Q  *  K  L  S  L  H  Q  M

5401  GTGTTGATATGAAAATATAATACTTTATACCATTAAATTGGTCTAGTAAGAATCATCCTC
1801   V  L  I  *  K  Y  N  T  L  Y  H  *  I  G  L  V  R  I  I  L

5461  ACGGATGGTTCTTTTTAGTTTCGCCGTTTGTAAAATTAAGTTAGAAAAAATAAAAAGCCA
1821   T  D  G  S  F  *  F  R  R  L  *  N  *  V  R  K  N  K  K  P

5521  TTTGTGATAGACTTTTGAGTATCCCTAATCAAAAGAAAGGCAATCACAAATGACCTATAA
1841   F  V  I  D  F  *  V  S  L  I  K  R  K  A  I  T  N  D  L  *

5581  ACATCTTACCACACGCGAATTAACTCTCATAGCTGATTTTTGGTATCAAGGCACTAAAGC
1861   T  S  Y  H  T  R  I  N  S  H  S  *  F  L  V  S  R  H  *  S

5641  TTATCGGGCTGCTAAATTACTTCAACGTAGTCAAGAAACCATCTATCGTGTTTATCGTTT
1881   L  S  G  C  *  I  T  S  T  *  S  R  N  H  L  S  C  L  S  F

5701  CCTCAATAACGGTAAAACCATCGACCAATATCTTCAGACTTATCAGCGACATAAACGTCG
1901   P  Q  *  R  *  N  H  R  P  I  S  S  D  L  S  A  T  *  T  S

5761  TTGTGGTCGGAAGCAGACCCAACTGCCAACTATCGAGGTTAACTATATCCATGCGCAAAT
1921   L  W  S  E  A  D  P  T  A  N  Y  R  G  *  L  Y  P  C  A  N

5821  CAAGGCTGGTTGGACTCCTGATACTATTATTGGTCGTGATGAGCACCCGATTAGCTGCAG
1941   Q  G  W  L  D  S  *  Y  Y  Y  W  S  *  *  A  P  D  *  L  Q

5881  ATACTAATGCTGATCAGCCAGCTCAAACAGCTGATAAAAATCAAGCAGCATCAAATGACA
1961   I  L  M  L  I  S  Q  L  K  Q  L  I  K  I  K  Q  H  Q  M  T
```

```
5941  CTACTAACCAAAGTAAAACTGATAGTACTTCAACAACTGGTAAGAATCTTACTTCTACAC
1981   L  L  T  K  V  K  L  I  V  L  Q  Q  L  V  R  I  L  L  L  H

6001  CAGTTTTCTACTTTGGCATCAACTGATAATGGAAAACAAAATCAAAATTATAATAAGCAT
2001   Q  F  S  T  L  A  S  T  D  N  G  K  Q  N  Q  N  Y  N  K  H

6061  GATAT
2021   D
```

SEQ ID No. 15 DNA
SEQ ID No. 16 PRT
Lactobacillus reuteri strain ML1 (ML4)

```
1     AATATTGATGGTTACTTAAGTTATACTGGTTGGTATCGTCCTTATGGAACGAGTCAAGAT
1      N  I  D  G  Y  L  S  Y  T  G  W  Y  R  P  Y  G  T  S  Q  D

61    GGTAAAACATGGTACGAAACAACTGCAATGGATTGGCGTCCATTACTGATGTATATTTGG
21     G  K  T  W  Y  E  T  T  A  M  D  W  R  P  L  L  M  Y  I  W

121   CCAAGCAAAGATGTTCAAGCACAATTTATTAAGTATTTTGTTAATAATGGTTATGAGAAT
41     P  S  K  D  V  Q  A  Q  F  I  K  Y  F  V  N  N  G  Y  E  N

181   GCTAATTATGGACTTACAGAGTCCTCTGTTGCTTCCTTTAGCAAGGATACTAATGCTAAT
61     A  N  Y  G  L  T  E  S  S  V  A  S  F  S  K  D  T  N  A  N

241   CTCCTCGATGTAACTGCACAAAATCTTCGTTATGTAATTGAGCAAAGTATTGCAGCCAAT
81     L  L  D  V  T  A  Q  N  L  R  Y  V  I  E  Q  S  I  A  A  N

301   AAAGGGACAAGTAAGTTAGCAAATGATATTAATAGTTTTGCTGCAACGGTTCCTGAATTA
101    K  G  T  S  K  L  A  N  D  I  N  S  F  A  A  T  V  P  E  L

361   TCTGCATCATCTGAATTATCATTGCAAAGCATGCCAAACTATCGACCAGATGAAAGTGGA
121    S  A  S  S  E  L  S  L  Q  S  M  P  N  Y  R  P  D  E  S  G

421   ACTGTTGATAGTGATCAAGTCATTTTTGTTAATAATAATTCAAAGGATCCCCGTAAAGGG
141    T  V  D  S  D  Q  V  I  F  V  N  N  N  S  K  D  P  R  K  G

481   AACACTGGTTATGCGGACAGCAACTATCGCTTAATGAACAGGACGATTAATAATCAGGCC
161    N  T  G  Y  A  D  S  N  Y  R  L  M  N  R  T  I  N  N  Q  A

541   GGAAATAATAATAGTGATAACAGTCCAGAACTCCTTGTTGGTAATGATATTGATAATTCA
181    G  N  N  N  S  D  N  S  P  E  L  L  V  G  N  D  I  D  N  S

601   AACCCAGTAGTACAAGCTGAAAATCTTAATTGGGAATACTTTTTACTAAATTATGGTAAG
201    N  P  V  V  Q  A  E  N  L  N  W  E  Y  F  L  L  N  Y  G  K

661   TTAATGGGGTATAATCCAGACGGTAATTTTGATGGCTTCCGAGTTGATGCTGCTGATAAT
221    L  M  G  Y  N  P  D  G  N  F  D  G  F  R  V  D  A  A  D  N

721   ATTGATGCAGATGTCTTAGATCAAATGGGTCAATTAATGAACGACATGTATCATACAAAG
241    I  D  A  D  V  L  D  Q  M  G  Q  L  M  N  D  M  Y  H  T  K

781   GGAAATCCTCAAAATGCAAATGATCATTTGAGTTATAATGAAGGTTATCATTCTGGGGCT
261    G  N  P  Q  N  A  N  D  H  L  S  Y  N  E  G  Y  H  S  G  A

841   GCACAAATGCTAAATGAAAAGGGTAATCCTCAATTGTACATGGATTCAGGCGAATTCTAT
281    A  Q  M  L  N  E  K  G  N  P  Q  L  Y  M  D  S  G  E  F  Y

901   ACCCTTGAGAATGTTCTCGGACGTGCAAATAACCGTGATAGTATCGGTAATTTAATTACT
301    T  L  E  N  V  L  G  R  A  N  N  R  D  S  I  G  N  L  I  T
```

```
961   AATAGTGTTGTTAATCGGCAAAATGATACAACAGAGAATGAAGCTACGCCAAACTGGTCA
321    N  S  V  V  N  R  Q  N  D  T  T  E  N  E  A  T  P  N  W  S

1021  TTTGTAACTAACCATGATCAACGAAAGAATTTGATTAATAGATTAATTATTAAGGGTCAT
341    F  V  T  N  H  D  Q  R  K  N  L  I  N  R  L  I  I  K  G  H

1081  CCTAACATTCCGGATATTATGGGTTCAGCTTACAAAGCTGAATATGCAAATCAAGCATGG
361    P  N  I  P  D  I  M  G  S  A  Y  K  A  E  Y  A  N  Q  A  W

1141  CAAGAATTCTACGCTGATCAGAAAAAGACTAATAAACAATATGATCAATATAATGTTCCG
381    Q  E  F  Y  A  D  Q  K  K  T  N  K  Q  Y  D  Q  Y  N  V  P

1201  GCTCAGTATGCAATTCTTTTGAGCAATAAAGATACGGTTCCGCAGGTTTACTATGGTGAC
401    A  Q  Y  A  I  L  L  S  N  K  D  T  V  P  Q  V  Y  Y  G  D

1261  CTTTATAATGAAACTGCTCAATACATGCAAGAGAAGTCAATTTACTATGATACAATCACG
421    L  Y  N  E  T  A  Q  Y  M  Q  E  K  S  I  Y  Y  D  T  I  T

1321  ACTCTTATGAAGGCCCGTAAACAATTTGTTAGTGGTGGTCAAACGATGACTAAACTTAAC
441    T  L  M  K  A  R  K  Q  F  V  S  G  G  Q  T  M  T  K  L  N

1381  AATAATTTATTAGCTAGTGTTCGATATGGTAAGGGTGTTGCTGATTCTAATAGCAATGGT
461    N  N  L  L  A  S  V  R  Y  G  K  G  V  A  D  S  N  S  N  G

1441  ACCGATAAGCTTAGCCGAACAAGTGGGATAGCCGTCTTAGTTGGTAATGATAGTAATATG
481    T  D  K  L  S  R  T  S  G  I  A  V  L  V  G  N  D  S  N  M

1501  GCTCAACAAACTGTTGCTATTAATATGGGTCGCGCTCATGCTAACCAACAATATCGAAAT
501    A  Q  Q  T  V  A  I  N  M  G  R  A  H  A  N  Q  Q  Y  R  N

1561  TTAATTGATACTACCGAAAATGGCTTGACATATGATGGAGAAAATAGTGAAAATCCAGCC
521    L  I  D  T  T  E  N  G  L  T  Y  D  G  E  N  S  E  N  P  A

1621  ATTTTGACAACTGATAGTAATGGTATCTTAAAAGTAACAGTTAAAGGATACAGTAACCCA
541    I  L  T  T  D  S  N  G  I  L  K  V  T  V  K  G  Y  S  N  P

1681  TACGTAAGTGGTTATCTTGGTGTTTGGGTTCCAGTAATTTCTGGTGATCAAGATGTTACT
561    Y  V  S  G  Y  L  G  V  W  V  P  V  I  S  G  D  Q  D  V  T

1741  ACAAGTGCAAGTGATGTTGTTGCTGATAAAGAAAAGACTTTTGAATCTAATGCTGCTCTT
581    T  S  A  S  D  V  V  A  D  K  E  K  T  F  E  S  N  A  A  L

1801  GATTCTCATATGATCTATGAAGATTTCAGCTTGTTCCAACCAGAACCAACTAATGTTGAG
601    D  S  H  M  I  Y  E  D  F  S  L  F  Q  P  E  P  T  N  V  E

1861  AATCATGCTTACAATGTGATTGCTAAAAATGCTAATCTCTTTAATGATTTAGGCATTACT
621    N  H  A  Y  N  V  I  A  K  N  A  N  L  F  N  D  L  G  I  T

1921  GATTTTTGGATGGCTCCTGCTTACACTCCATTTGGAATGAGTCGTTATAATGAAGGATAC
641    D  F  W  M  A  P  A  Y  T  P  F  G  M  S  R  Y  N  E  G  Y

1981  TCAATGACGGATCGTTACAATTTAGGTACGACAGCTAATCCAACAAAGTATGGTAGTGGA
661    S  M  T  D  R  Y  N  L  G  T  T  A  N  P  T  K  Y  G  S  G

2041  GAAGAGCTTGCAAATACAATTGCTGCATTGCATAAAGTAGGATTAAAAGTTCAAGAAGAT
681    E  E  L  A  N  T  I  A  A  L  H  K  V  G  L  K  V  Q  E  D

2101  ATTGTTATGAATCAGATGATTGGTTTCTCTGGTCAAGAAGCAGTAACGGTTACTCGAACA
701    I  V  M  N  Q  M  I  G  F  S  G  Q  E  A  V  T  V  T  R  T

2161  AATAATCGTGGAATGCAGATTCATGTAAATGGTCAAACATATGCAAATCAAATTTACTTT
721    N  N  R  G  M  Q  I  H  V  N  G  Q  T  Y  A  N  Q  I  Y  F
```

```
2221  GCATATACAACTGGTGGCGGAAATGGTCAAGAAACTTATGGTGGTAAATACCTTGCCGAA
741    A  Y  T  T  G  G  G  N  G  Q  E  T  Y  G  G  K  Y  L  A  E

2281  TTACAAAAGAACTATCCTGACCTATTTACGACCAAGGCAATTTCGACAGAAGTTGTACCT
761    L  Q  K  N  Y  P  D  L  F  T  T  K  A  I  S  T  E  V  V  P

2341  GATCCAACCGTTCGTATTAAT
781    D  P  T  V  R  I  N
```

SEQ ID No. 17 DNA
Lactobacillus strain LB33

```
1     ATGGAATTAA AAAGGCATTA CAAGATGTAC AAGGCTGGTA AAAAATGGGT TTTTGCTGCA
61    ATTGCCACAA TCTCTATAAT TGCAGGATTA AATACAGTGG CAGTGACAAC CTATGCTGCC
121   GGCAATAATG ATCCGCAGCA GACCACTACT CAAAATGCAC CTAACAACAG TAACGATCCG
181   CAATCTACTA CTACGCAGAA TACTGCCAAC AACAGTAACG ATCCGCAATC TACTACTACG
241   CAGAATACTG CCAACAACAG TAATGGTCCA CAATCTACTA CTACGCAGAA TACTGCCAAC
301   AATAGTAATG GTCCACAATC TACTACTACG CAGAATACTG CCAATAACAG TAACGATCCA
361   CAATCTACTA CTACGCAGAA TACTGCCAAC AACAGTAACG ATCCGCAATC TACTACTACG
421   CAGAATACTG CCAACAATAG TAATGGTCCA CAATCTACTA CTACGCAGAA TACTGCCAAC
481   AACAGTAACG ATCCGCAATC TACTACTACG CAAAACACTG CCAACAACGG TAATGATCCA
541   CAATCTACTA CTGGAAAAGA TACAGTTAGT ATTGCAGATA TTCAAGTTAA CCAACCTGTT
601   AATCTTTTAG GAAAGCAATC AACTGTATCT AGTACTGGTT ATAATGACTC TCACATAAAA
661   AATGTCAATG GGAAAATCTA TTTTGTTGGT GATAATGGTC AGGTCAAGAA AAACTTTACA
721   GCCATAATCA ATGGACAATC ACTATATTTC AATAAAACAA CTGGAGAATT GGCTTCTAAT
781   GATGTTCAAT ATGAAAATGG GTTAGTAAAA ATAAACGATG TTCATAACGC CGCTTACTCT
841   ATTGATCCAA CGGGATTCAC TAATGTTAAC GGATTTTTAA CTGCTAATAG TTGGTATAGA
901   CCCAAATATA TTTACAAAGA TGGGCAAAAA TGGGTGGAAT CAACCTCTCA AGATATGCGT
961   CCCCTTTTAA TGACATGGTG GCCAGATAAA AATACTCAAG TAGCTTATTT ACAATATATG
1021  CAGAAAATGG GCATTTTACC CGCTGACGTC ACTATATCAA GTCAAACCAA TCAATCAGTT
1081  TTAACCAAAG AATCATTTAT TACTCAAGCT GAAATTGAAA AACAGATTGG AGTAACAAAT
1141  GGAAACACTG ATTGGCTAAA GAAAGATATC TCTGATTTTG TAAATTCTCA ACCAAATTGG
1201  AATATAGATA GTGAAGCCAA AGGCACAGAC CATTTGCAGG GGGGAGCACT TTTATATGTT
1261  AATAATAAGT TAACTCCATA TGCGAATTCT GATTACCGCT TGCTTAACCG AACACTTACT
1321  AATCAACAGG GGCAAGTAAA AGATACTTCT AAACAAGGCG GTTATGAAAT GTTACTTGCC
1381  AACGATGTGG ATAATTCCAA TCCAGTAGTT CAAGCGGAAC AGTTAAACTG GTTATACTAC
1441  ATGATGAATA TAGGTAGCAT TACTGCCAAT GATCCCACCG CAAACTTTGA TGGCTATCGA
1501  GTGGACGCTG TGGACAATGT CGATGCTGAT TTATTAAATA TAGCTGCCGA TTATGCCAAA
1561  GATGCTTATA AAACTAATCA AAGTGATGCT AATGCCAACA AACATTTATC AATATTAGAA
1621  GATTGGGATA ATAATGATCC GGCTTATATC AAAGCACATG GAAATAATCA GTTAACTATG
1681  GATTTCCCAG CACATTTAGC AATTAAATAT TCATTAAATA TGCCAGTAAG TCAACGAAGT
1741  GGGCTGGAAC CAGAGCTCAC AACCAGTTTA GTTAACAGAA CTGGTGATGA TTCTACTGAA
1801  AATGTCGCAC AGCCAAACTA TACTTTTATT AGGGCTCACG ATAGTGAAGT GCAAACAATC
1861  ATCGCACAAA TTATCAAAGA TAAAATCAAC CCTAACTCTG ACGGATTAAC AGTTACTCCC
1921  GATGAAATAA GTCAGGCCTT TAAAATATAT AATGCAGATG AATTAAAGAC TGATAAACAA
1981  TATACTTTTT ATAACATGCC CTCTGCCTAT ACTATTTTGC TAACCAATAA AGATACAGTA
2041  CCTCGAGTTT ATTATGGGGA TCTTTATAGT GATAATGGCA ATTATATGTC AGCCCATTCT
2101  CCTTACTATG ATGCAATAAC TACGTTATTA AAAACACGAA TGAAATACGT ATCTGGTGGT
2161  CAAAACATGC GTATGCAATA TATGCAGGGT GATGATATGC CTGCTAATAG CTATAAGGGC
2221  GTTTTAACTT CAGTTAGATA TGGTAAGGGT GAAATGACAG CCGATGAGCA AGGTAATTCA
2281  GAAACTCGTA CTCAAGGAAT TGGGGTCATT ATAAGCAATA ATCCTAATTT AAAATTAGAC
2341  AGTAATGACC AAGTGGTATT AAATATGGGG GCGGCACATG AAAATCAAAC TTATCGCCCT
2401  GTATTACTAA CAACTAAAGA TGGATTGAAA AACTATGATT CCGATAGTTC TGTACCTCAA
2461  AATGCATTAG TTTCAACCAA CGATAAGGGA CAACTCATAT TTAAAGCTAG TTCTATTCAG
2521  GGAGTAAGTA ATCCGCAGGT ATCTGGTTAT TTGTCCGTGT GGGTCCCAGT GGGGGCAAAG
2581  GATAATCAAG ATGCTCGGAC TGCAAGCAGT TCTCAGCCAT CAACTGATGG GAAAACATAT
2641  CATTCCAATG CTGCTTTAGA CTCTCAAGTT ATTTACGAAG GATTTTCTAA TTTTCAATCG
2701  ATTCCTACAA ATACAGAAGA TTTCACTAAT GTAAAAATTG CTCAAAACGC TAACCTGTTT
2761  AAGAGCTTGG GAATAACAAG TTTTGAATTA GCCCCTCAAT ATCGTTCCAG TAATGATAAT
2821  AGTTTTCTGG ATTCGGTTGT TCAAAATGGC TACGCATTTA CTGATCGTTA TGATATTGGG
2881  TATAATACTC CGACAAAATA TGGAACTGTT ACTCAATTGC TGGATGCATT AAGGGCTTTA
2941  CATGCCAACG GAATTCAAGC GATCGATGAC TGGGTTCCTG ACCAAATATA CAATTTACCT
3001  GGTGAGGAAA TTGTCGCAGC TCAAAGAACT AATGGATCTG GGACATATGA TCAAGATTCT
```

```
3061  GTTATTGATG ATACATTATA TGATTCTCAC ACTGTTGGTG GTGGCGAATA TCAAGCTAAA
3121  TTTGGTGGAG CTTTTCTAAA CAAGTTAAAG CAGTTGTATC CTGATTTATT TAAAGTTAAA
3181  CAAATTTCTA CTGGTCAACC TATGAATCCT AATGAAAGAA TTACCGAGTG GTCAGCAAAG
3241  TACTTTAATG GTACAAATAT TCAAGGAAGA GGCGCTTGGT ATGTATTAAA AGACTGGGGT
3301  ACCAATCAGT ACTTTAATGT AAGTAATAAC CAGTTTGTTC CCAAACAATT CCTAGGTACA
3361  GATACTTATA CAGGCTTTAA TGTTACAAAT GAGGGAACTC AGTTTTATTC TACGAGTGGG
3421  TATAAAGCCC AGAATACCTT TATTCAGGAC GGAGACAACT GGTATTACTT TGACAATAAT
3481  GGCTATATGG TAACTGGTTT ACAGAATATA AATGGGAATA ATTACTATTT CTTGCCCAAT
3541  GGCATTGAAC TACAAGACTC TTATTTATTG AATGATGATA CCGGTAAAGA ATATTATTAT
3601  GCAAGTAATG GTAAGCAAAT CTCAAATCGT TATTATCCAG ATGCTAACGG CAATTGGAGA
3661  TATTTCTTCA ATGATGGTTC AATGGCAAGA AATGGATTAA CCACTATTGA ACAACCAGAT
3721  GGGCAAAAAG TGATCCAATA TTTTGATTCC GATGGTATTC AATTAAAGGG AAATGCCGCA
3781  AAAGATAATA ATGGTAATTT AAGATATTTT GACGGTAATA CAGGTGATAT GGTCATTAAT
3841  TCATTTGGAG AACTTCCTGA TGGCTCTTGG TTATACCTTA ATGATAAGGG GATTGCCGTT
3901  ACTGGTAAAC AGGAAATCAA TGGTCAAACC TACTACTTTG ATGCGGATGG CAAGCAAGTG
3961  AAGAATGATT TTAGAGAGTT GCCTGATGGT TCATGGCTTT ATCTTAATGA CAAGGGGATT
4021  GCCGTTACTG GTAAACAGGA AATCAATGGT CAAACCTACT ACTTTGATGC GGATGGCAAG
4081  CAAGTGAAGA ATGATTTTAG AGAGTTGCCT GATGGTTCAT GGCTTTATCT TAATGACAAG
4141  GGGATTGCCG TTACTGGTAA ACAGGGAATC AATGGTCAAA CCTATGCAGA GGCTAAAATC
4201  ACAGCTGCCG AAAATGCTCA TCAAGCTGCC ACAGACGCTG TGAATAAAGC CCAAGCTGCT
4261  CAATCGCCTA ACACTAGTTC CTCTAGTTCT AGCGTTAGCC AAGCTACTAA ACATCAATTG
4321  GCAGTTAAAA CTGCTAAAGC TCAACTTGCT AAAACTAAGG CTCAAATTGC TAAGTATCAA
4381  AAGGCTTTGA AAAAAGCCAA AACTACAAAG GCCAAGGCTC AAGCTCGTAA AAGTTTGAAG
4441  AAGGCCGAGA CTAGTTTCAG CAAAGCTGAA CTTAATTTGG CATTATTAAA TAATAAAGCC
4501  GTAAAAGCTG CACAAACTAA GGTTAATAAG GCTAAGGCTC AAGTCACTAA ATACCAAAAG
4561  GCTTTGAAGA AAGCTAAGAC TACAAAGGCT AAGACTCAAG CTCGTAAAAA TTTGAAGAAG
4621  GCCAACTCTA GTCTGACAAA AGCTCAAAAA GCATTAACTA AAGTAATTAA AACCAATATC
4681  AAGTAA
```

SEQ ID No. 18 PRT
Lactobacillus strain LB33

```
MELKRHYKMYKAGKKWVFAA IATISIIAGLNTVAVTTYAA
GNNDPQQTTTQNAPNNSNDP QSTTTQNTANNSNDPQSTTT
QNTANNSNGPQSTTTQNTAN NSNGPQSTTTQNTANNSNDP
QSTTTQNTANNSNDPQSTTT QNTANNSNGPQSTTTQNTAN
NSNDPQSTTTQNTANNGNDP QSTTGKDTVSIADIQVNQPV  200
NLLGKQSTVSSTGYNDSHIK NVNGKIYFVGDNGQVKKNFT
AIINGQSLYFNKTTGELASN DVQYENGLVKINDVHNAAYS
IDP?GFTNVNGFLTANSWYR PKYIYKDGQKWVESTSQDMR
PLLMTWWPDKNTQVAYLQYM QKMGILPADVTISSQTNQSV
LTKESFITQAEIEKQIGVTN GNTDWLKKDISDFVNSQPNW  400
NIDSEAKGTDHLQGGALLYV NNKLTPYANSDYRLLNRTLT
NQQGQVKDTSKQGGYEMLLA NDVDNSNPVVQAEQLNWLYY
MMNIGSITANDPTANFDGYR VDAVDNVDADLLNIAADYAK
DAYKTNQSDANANKHLSILE DWDNNDPAYIKAHGNNQLTM
DFPAHLAIKYSLNMPVSQRS GLEPELTTSLVNRTGDDSTE  600
NVAQPNYTFIRAHDSEVQTI IAQIIKDKINPNSDGLTVTP
DEISQAFKIYNADELKTDKQ YTFYNMPSAYTILLTNKDTV
PRVYYGDLYSDNGNYMSAHS PYYDAITTLLKTRMKYVSGG
QNMRMQYMQGDDMPANSYKG VLTSVRYGKGEMTADEQGNS
ETRTQGIGVIISNNPNLKLD SNDQVVLNMGAAHENQTYRP  800
VLLTTKDGLKNYDSDSSVPQ NALVSTNDKGQLIFKASSIQ
GVSNPQVSGYLSVWVPVGAK DNQDARTASSSQPSTDGKTY
HSNAALDSQVIYEGFSNFQS IPTNTEDFTNVKIAQNANLF
KSLGITSFELAPQYRSSNDN SFLDSVVQNGYAFTDRYDIG
YNTPTKYGTVTQLLDALRAL HANGIQAIDDWVPDQIYNLP 1000
GEEIVAAQRTNGSGTYDQDS VIDDTLYDSHTVGGGEYQAK
FGGAFLNKLKQLYPDLFKVK QISTGQPMNPNERITEWSAK
YFNGTNIQGRGAWYVLKDWG TNQYFNVSNNQFVPKQFLGT
DTYTGFNVTNEGTQFYSTSG YKAQNTFIQDGDNWYYFDNN
GYMVTGLQNINGNNYYFLPN GIELQDSYLLNDDTGKEYYY 1200
ASNGKQISNRYYPDANGNWR YFFNDGSMARNGLTTIEQPD
```

GQKVIQYFDSDGIQLKGNAA KDNNGNLRYFDGNTGDMVIN
SFGELPDGSWLYLNDKGIAV TGKQEINGQTYYFDADGKQV
KNDFRELPDGSWLYLNDKGI AVTG?QEINGQTYYFDADGK
QVKNDFRELPDGSWLYLNDK GIAVTGKQGINGQTYAEAKI 1400
TAAENAHQAATDAVNKAQAA QSPNTSSSSSSVSQATKHQL
AVKTAKAQLAKT?AQIAKYQ KALKKAKTTKAKAQARKSLK
KAETSFSKAELNLALFK

SEQ ID No. 19 DNA
SEQ ID No. 20 PRT
Lactobacillus sake strain KG15

```
1      SASCTGBCMSTNACGTTHRRCNTAGACGTTHRACGTACTGGTTCACACAATGGATTCGGC
1        X  X  X  X  R  X  X  X  T  X  X  V  L  V  H  T  M  D  S  A

61     AAACTATCAATGATTGCGATCTGTCCAGGTTGGGCTGCTTCACGCGTCAAACCAGTACGG
21       N  Y  Q  *  L  R  S  V  Q  V  G  L  L  H  A  S  N  Q  Y  G

121    ATCGCATTGACCACGGGTAATAATTGTAGTGCGCGACGGTTGAACCGTGACCGACTAATG
41       S  H  *  P  R  V  I  I  V  V  R  D  G  *  T  V  T  D  *  W

181    GTGATTTTTTGCGGCATAAAGGCGGTCATCAAGCGCCAAAAACGGCGTTGTGATTGAATA
61       *  F  F  A  A  *  R  R  S  S  S  A  K  N  G  V  V  I  E  Y

241    CCAAGCGTTGTTTGTAAACACAGTAGCGCCAACAATCGACAGTCATCGATTTTAACGTGC
81       Q  A  L  F  V  N  T  V  A  P  T  I  D  S  H  R  F  *  R  A

301    GCCACATTACGCCGTTGCGTCACACAACGTGGGCAATAGCGCTGGTAAAGCGACTGGCAC
101      P  H  Y  A  V  A  S  H  N  V  G  N  S  A  G  K  A  T  G  T

361    AGCTGATAAAAATAATGATAGTTACCTTGTAATTCGTGACGAATTTGTTTAAACTTAGGA
121      A  D  K  N  N  D  S  Y  L  V  I  R  D  E  F  V  *  T  *  D
                                                          - 35
421    TGGTTCAACATCGTTAGGACCCCTTTTAAGTTTAGTCACTTATGAATCTAACTGTGTTGG
141      G  S  T  S  L  G  P  L  L  S  L  V  T  Y  E  S  N  C  V  G
                              - 10
481    ACTTTTTTGTTAATTTTTTTGTATTATTACAAACTAGCACCACGCGTATGTGTTTTATTA
161      L  F  C  *  F  F  C  I  I  T  N  *  H  H  A  Y  V  F  Y  *
                                                       RBS
541    ATACCACTTAATTAATAACGGGGCTTTAGCATGATTTCAAATAAAATAGTGTGAAAGGTA
181      Y  H  L  I  N  N  G  A  L  A  *  F  Q  I  K  *  C  E  R  *
            start
601    GTTTTTTATGTTAAGGAATAATTATTTTGGAGAGACTAAAACGCATTATAAATTATATAA
201      F  F  M  L  R  N  N  Y  F  G  E  T  K  T  H  Y  K  L  Y  K

661    ATGCGGTAAGAACTGGGCTGTCATGGGGATTTCATTATTTCCGCTGGGATTAGGGATGCT
221      C  G  K  N  W  A  V  M  G  I  S  L  F  P  L  G  L  G  M  L

721    AGTTACCAGCCAGCCAGTGTCAGCTGATGTGACAGCCACCAGCACCTCAAGCAGTGCAGT
241      V  T  S  Q  P  V  S  A  D  V  T  A  T  S  T  S  S  S  A  V

781    GAGGACCGATGCAATCAGTGCAAGTAGTAGCAGTGCAGCAAAGGCTGAAACGGCTGCGAT
261      R  T  D  A  I  S  A  S  S  S  S  A  A  K  A  E  T  A  A  I

841    CACTACTGCAGGTGTTGCAAATGCTGATTCACAAACATCAGCAGAAGTAACCGCTGACTC
281      T  T  A  G  V  A  N  A  D  S  Q  T  S  A  E  V  T  A  D  S

901    TACTTCTACCAGCCAAGTGGTAACTAATAATTCCAATAATCAAAATAATACAGCACAGCC
301      T  S  T  S  Q  V  V  T  N  N  S  N  N  Q  N  N  T  A  Q  P

961    AGCCGGTCAAGAAGCAGCCCCGGTATCAGAGGACACATCATCTGATGATAGTGAGAGAAC
321      A  G  Q  E  A  A  P  V  S  E  D  T  S  S  D  D  S  E  R  T
```

```
2281  TCAGGGTGTTATGGTAACGGGTAAGCAACGTGTGCACCAAGATCAGTATTTCTTCCTGCC
761     Q  G  V  M  V  T  G  K  Q  R  V  H  Q  D  Q  Y  F  F  L  P

2341  AAATGGTATTGCTTTGACAGATGCTTTCGTACAAACTGCTGATGGTCAACGTCAGTACTA
781     N  G  I  A  L  T  D  A  F  V  Q  T  A  D  G  Q  R  Q  Y  Y

2401  TGATAAAACAGGTCGTCTGGTCATTAATCAATATGTGACTGACCACCAAGCGAATGCGTT
801     D  K  T  G  R  L  V  I  N  Q  Y  V  T  D  H  Q  A  N  A  F

2461  CCGGGTTGATGCAGACGGTAACGTTGTCCGCAATCAAGCTTTGACTGTTGACGGCCATGA
821     R  V  D  A  D  G  N  V  V  R  N  Q  A  L  T  V  D  G  H  E

2521  ACAATATTTCGGCACAAACGGTGTCCAAGCGAAAGCAGTGCTCATTCGAACTGACGATAA
841     Q  Y  F  G  T  N  G  V  Q  A  K  A  V  L  I  R  T  D  D  N

2581  TCAGGCGCGCTACTACGAAGCCAATAGTGGTAATCTCGTGAAGCAACAGTTTATTCTTGA
861     Q  A  R  Y  Y  E  A  N  S  G  N  L  V  K  Q  Q  F  I  L  D

2641  TACAGATGGACATTGGTTGTACGCGGATGCTGCAGGTGACTTGGCACGCGGACAAATTAC
881     T  D  G  H  W  L  Y  A  D  A  A  G  D  L  A  R  G  Q  I  T

2701  AATTGGCCAAGACACGTTGTATTTTGATGATAATAATCACCAGGTAAAAGATGATTTCGT
901     I  G  Q  D  T  L  Y  F  D  D  N  N  H  Q  V  K  D  D  F  V

2761  CTATGATACTAACGGTGTGCATTATTTTAATGGCACAACAGGCGCTGAAATCAAACAAGA
921     Y  D  T  N  G  V  H  Y  F  N  G  T  T  G  A  E  I  K  Q  D

2821  TTACGCGTTTCATGATGGCAAATGGTACTATTTTGATGATTTGGGACGAATGGTAACCGG
941     Y  A  F  H  D  G  K  W  Y  Y  F  D  D  L  G  R  M  V  T  G

2881  CTTGCAGCGTATTAATGGTGAGTATCGCTATTTTGATGCTAATGGTGTGCAACTAAAGGG
961     L  Q  R  I  N  G  E  Y  R  Y  F  D  A  N  G  V  Q  L  K  G

2941  CGGTACCGTGACCGATCCACTAACGCACCAAACGTACACTTTTGATGCGAAAACTGGTGC
981     G  T  V  T  D  P  L  T  H  Q  T  Y  T  F  D  A  K  T  G  A

3001  TGGTACGTTGGTGACGATTTAACTGAATAATGGACTAGAAAAGACGATCTTGTATCGTCT
1001    G  T  L  V  T  I  *  L  N  N  G  L  E  K  T  I  L  Y  R  L

3061  TTTTTAGTTTCGATAACTAAATAAGTGCTCATTTTTGCATTAGGACTCAGAATTAGCGGG
1021    F  *  F  R  *  L  N  K  C  S  F  L  H  *  D  S  E  L  A  G

3121  CGCGCAAGCGTCTTTTCGTGTTAAACTTATTAGTAATTAATATTTTGAGGAGTCTGTTAT
1041    A  Q  A  S  F  R  V  K  L  I  S  N  *  Y  F  E  E  S  V  I

3181  ATGGCAACAATTTTAGTTGTAGATGATGAACCGTCATTGGTGACGCTACTGTCATACAAC
1061    W  Q  Q  F  *  L  *  M  M  N  R  H  W  *  R  Y  C  H  T  T

3241  CTGACTAAATCAGGCTTCGAGGTCGTGACTGCTACCTCCGGTGACGAGGCACGAAATCAG
1081    *  L  N  Q  A  S  R  S  *  L  L  P  P  V  T  R  H  E  I  S

3301  CTGGCAAATCATCCTATTGATTTGATGCTGCTAGGTGTCATGTTGCCTGGTAAGAGTGGC
1101    W  Q  I  I  L  L  I  *  C  C  *  V  S  C  C  L  V  R  V  A

3361  GTTGACTTAACACGAGAACTACGAGGCGAACAGAATCGTATTCCAATTATTATGATTACC
1121    L  T  *  H  E  N  Y  E  A  N  R  I  V  F  Q  L  L  *  L  P

3421  GCCTTGGATGACGAAGTTGACAAGATTT
1141    P  W  M  T  K  L  T  R  F
```